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# Ontogeny of the digestive tract in butter catfish *Ompok bimaculatus* (Bloch) larvae

**P. K. Pradhan · J. K. Jena · G. Mitra · N. Sood · E. Gisbert**

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**Abstract** The ontogeny of the digestive tract was studied histologically in butter catfish *Ompok bimaculatus* from hatching to 30 days post-hatching (dph). At hatching, the digestive tract of butter catfish consisted of a straight tube with a smooth lumen dorsally attached to the yolk sac. Between 1 and 2 dph, the mouth opened, oral valves were visible and canine-like teeth and taste buds were detected. During this period, intestine was differentiated into the anterior and posterior intestine, and the digestive accessory glands were also developed. Exogenous feeding started at 2 dph, 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. Between 3 and 4 dph, several vacuoles (neutral lipids) were observed in the intestine and also in hepatocytes, indicating a functional absorption of nutrients from food. At 8 dph, differentiation of gastric glands was noticed, and by 9–11 dph, there were abundant gastric tubular glands arranged along numerous longitudinal folds. During the same period, pyloric sphincter appeared as an epithelial fold that separated the stomach from the anterior intestine. From 12 dph to the end of the study at 30 dph, no noticeable histological modifications were observed. The development of gastric glands is considered as the last major events in digestive tract development and their presence designates the end of larval period and the onset of the juvenile period. Hence, it is suggested that, butter catfish larvae have a morphologically complete digestive tract by 12 dph. These findings on the development of the digestive system in butter catfish may lead to a better understanding of the ontogeny and would be useful to improve the larval rearing techniques of this promising catfish species for freshwater aquaculture diversification.

**Keywords** Ontogeny · Larvae · Digestive tract · Histology · Butter catfish

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## Introduction

Butter catfish *Ompok bimaculatus* (Bloch), a fish species belonging to the family of Siluridae, has a very high consumer preference in the north-eastern and eastern states and also in the mainlands of India. The species is a delicacy for the people of the region and it

is considered as the costliest food fish ($8–12/kg). Thus, this species is considered as an important candidate for the diversification of freshwater Indian aquaculture. Further, the species is categorized as an endangered fish species according to the International Union for Conservation of Nature and Natural resources (IUCN) criteria and has been facing high risk of extinction in the wild (CAMP 1997). The depletion of natural resources at an alarming rate warrants immediate action to arrest and reverse the trend at the earliest. Assured supply of the fish through aquaculture can also help in conservation of the species through reduction on fishing pressure. For successful culture, a reliable larval rearing technique must be developed to ensure consistent production of good quality fry. However, the high mortality rate of larvae during larval rearing is the most serious bottleneck at present for commercial production of this species.

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 food (Kjørsvik et al. 2004). Although fish larvae may be morphologically capable of capturing food items at first feeding (Segner et al. 1994), their digestive system needs a series of developmental changes before being fully functional (Govoni et al. 1986). 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 ontogeny (Treviño et al. 2011). 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 microscopical methods is probably the most accurate indicator of nutritional status of fish larvae (Gisbert et al. 2008). 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 (Zambonino-Infante et al. 2008).

Studies on the ontogenesis of the digestive system in some commercially important fish species have been carried out in recent years (see reviews in Zambonino-Infante et al. 2008; Lazo et al. 2011). Most of these studies have been focussed on marine fish species, but very little research has been done on freshwater aquaculture species, especially catfish (Verreth et al. 1992; Kozarić et al. 2008; de Amorim et al. 2009; Yang et al. 2010; Saelee et al. 2011). To date, no study has been documented on the ontogeny of the digestive system in butter catfish, 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 butter catfish culture still demands of 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 microdiet. Thus, in order to enhance the success of larval rearing of this catfish 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 butter catfish, from hatching to 30 days post-hatching (dph). This new information is expected to provide fundamental knowledge for improving actual larval rearing practices for this catfish species.

## Materials and methods

### Eggs and larval rearing

Fertilized eggs from the same batch of brood butter catfish were collected from Tripura State Government Hatchery at Agartala, Tripura (India). The eggs were produced through artificial breeding. Female butter catfish (44–49 g) were injected with Ovaprim® (1.0 ml $kg^{-1}$ body weight, BW) and male butter catfish (44–46 g) with Ovaprim® (0.5 ml $kg^{-1}$ BW). Between 9 and 10 h post-injection, females were stripped individually into dry enamel trays. Milt was obtained from the males by surgically removing their testes, which were macerated to produce a suspension to be mixed with the eggs for fertilization. Eggs were subsequently washed thoroughly with clean water and hatched in $3 \times 1 \times 0.60$ $m^3$ cement incubators at

27.0 ± 1.1 °C with mild aeration. After hatching (23 ± 1 h post-fertilization), the larvae were stocked in three fibreglass tanks (4 × 1 × 0.75 $m^3$) at the density of 5 larvae $l^{-1}$. Tanks were connected to a flow-through water system with an exchange rate of 1.2–2.0 l $min^{-1}$, where fish were reared until the juvenile stage (30 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 80$^{TM}$, Ocean Star International, Inc. USA) from mouth opening (2 dph) until 10 dph. From 7 dph onwards, zooplankton collected from nearby ponds, which consisted mainly of copepods (Cyclopoida), were also added to fish rearing tanks. After 10 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 27.0 ± 1.1 °C, 6–8 mg $l^{-1}$ and 6.8–7.6, respectively. Fish were held under natural photoperiod according to the rainy season of the year (25°53′ N, 91°55′ E).

### Larval sampling and growth measurements

A random sample of 30 larvae was daily collected from hatching (0 dph) to 15 dph and every third day from 18 to 30 dph from the mass rearing tanks. Larvae were killed with an overdose of tricaine methanosulphonate (MS-222, Sigma), rinsed in distilled water, measured in length and weight and fixed in neutral-buffered formaldehyde. Total length (TL) of 20 randomly selected larvae were individually measured using a scale to the nearest of 0.1 cm, and once larvae were measured in length, they were weighed to the nearest 0.001 g with an analytical microbalance (Mettler Toledo AG245). For histological purposes, ten larvae from each sampling day were dehydrated in a graded series of ethanol, embedded in paraffin and cut into serial sagittal sections (3–5 μm thick). Sections were stained by Harris' Haematoxylin and Eosin (HE) procedure for general histomorphological observations, while periodic acid-Schiff (PAS) was used to detect neutral glycoconjugates in mucous cells and glycogen deposits in the liver (Pearse 1985). Pigmented granules found during yolk sac absorption in the periphery of the yolk sac were identified according to Gisbert and Sarasquete (2000). The volume of intestinal and hepatic fat deposits (unstained vacuoles that corresponded to lipids dissolved during the embedding process of the larva in paraffin) was estimated according to the volume formula of a spheroid: $V = 4/3ð\ a^2b$, where $a$ and $b$ are the minimum and maximum diameters of the spheroid, respectively. Measurements on histological slides were based on the analysis of six randomly chosen fields in the liver on a total of five fish by means of an image analysis software package (ANALYSIS$^{TM}$, Soft Imaging Systems GmbH, Münster, Germany) and expressed as mean ± standard error.

## Results

### Larval growth

Butter catfish larvae showed exponential growth for wet weight and total length from hatching until the end of the study (30 dph) (Fig. 1).

### Histological development of digestive system

#### *Yolk sac*

At hatching (0.3 cm TL), the digestive tract of butter catfish consisted of a straight tube with a smooth lumen (primordial intestine), 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. 2a). Acidophilic yolk platelets that were in contact with the yolk syncytial layer (Jaroszewska and Dabrowski 2011) were smaller than those in the centre of the yolk sac matrix (Fig. 2b). Most of the yolk sac reserves were consumed between 2 and 3 dph (0.33 − 0.42 ± 0.04 cm TL), whereas yolk sac did represent only 10% of the initial size at 4 dph (0.45 ± 0.05 cm TL). At this age, yolk platelets were restricted to the anterior region of the abdominal cavity, close to the liver and heart (Fig. 5a). Remnants of yolk in contact with the venous circulation were surrounded by the yolk syncytial layer with scattered cells containing melanin pigment granules (bleached with hydrogen peroxide) that resembled macrophages (Fig. 2d). At 5 dph, the yolk sac was completely depleted and no longer visible in histological sections.

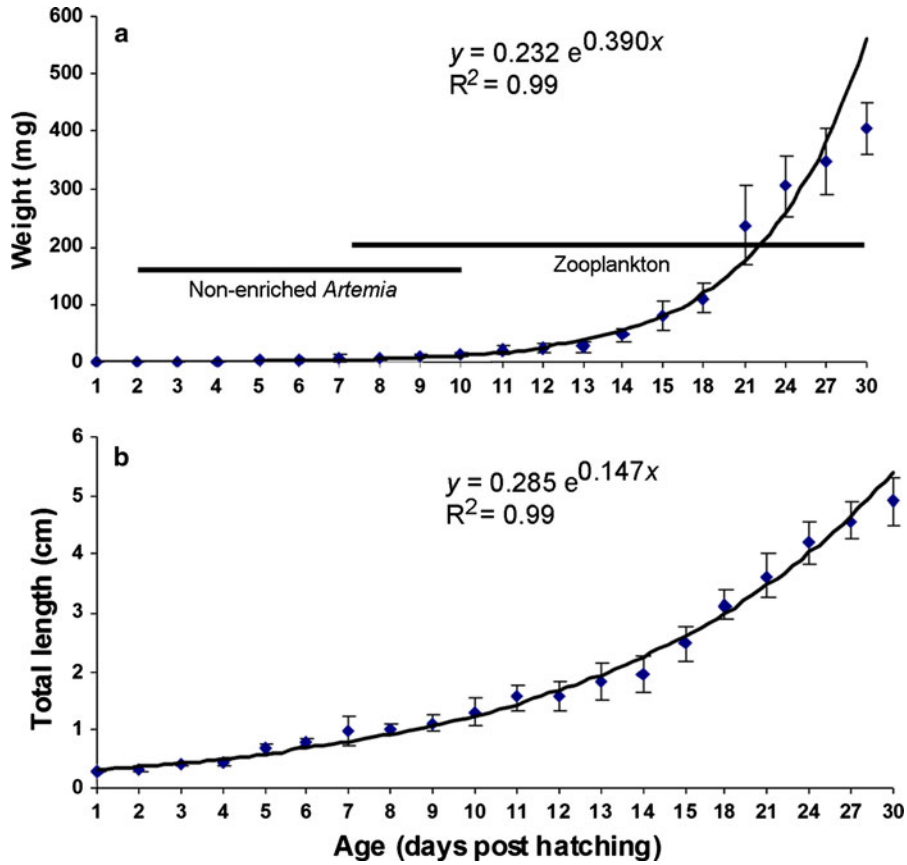



**Fig. 1** Growth in weight (g) and total length (cm) of butter catfish (*Ompok bimaculatus*) larvae from 1 to 30 dph. The feeding protocol for butter catfish is also included

*Mouth, oral cavity and pharynx*

Between 1 and 2 dph, the mouth opened, and two oral valves were clearly visible in the larvae (Fig. 3b). The buccopharyngeal epithelium was composed of a single layer of squamous cells (Fig. 3c) with scattered round mucous cells, mostly found in both oral valves. At 2 dph, the oral cavity was short, and canine-like teeth were detected in both oral valves (premaxilla and Meckel's cartilage) protruding into the buccopharyngeal lumen. Taste buds were detected on the basis of gill arches lining the pharyngeal cavity (Fig. 3b). Scattered round-shaped ionocytes were detected in both oral valves as well as in the pharyngeal epithelium (Fig. 3b, d). Clusters of muscular cells, which would constitute the future tongue, were found posterior to the ventral oral valve, just before the first pharyngeal pouch. Between 4 and 6 dph (0.45 ± 0.05 cm − 0.78 ± 0.07 cm TL), the size of the oral cavity increased, as well as the number of canine-like teeth (Fig. 3e) in both oral valves. The abundance of mucous cells increased, mostly in the posterior part of the pharyngeal lumen close to the oesophagus. Pharyngeal teeth developed in the connective tissue underlying the pharyngeal submucosa, protruding into the pharyngeal lumen between 6 and 7 dph (Fig. 3f). The number of oral and pharyngeal teeth, mucous cells and taste buds substantially increased at 9 dph (1.12 ± 0.13 cm TL). Pharyngeal papillae started to differentiate close to the connection of the pharynx with the oesophagus and grew in size along larval development. No noticeable histological changes were observed after 9 dph until the end of the study at 30 dph (4.69 ± 0.71 cm TL).

*Oesophagus*

Between 2 and 3 dph, the oesophagus started to differentiate in larvae as a short duct lined by a simple cuboidal epithelium (Fig. 4a) with very few mucous cells connecting the pharyngeal cavity with the anterior intestine (Fig. 4b). Between 3 and 6 dph, the

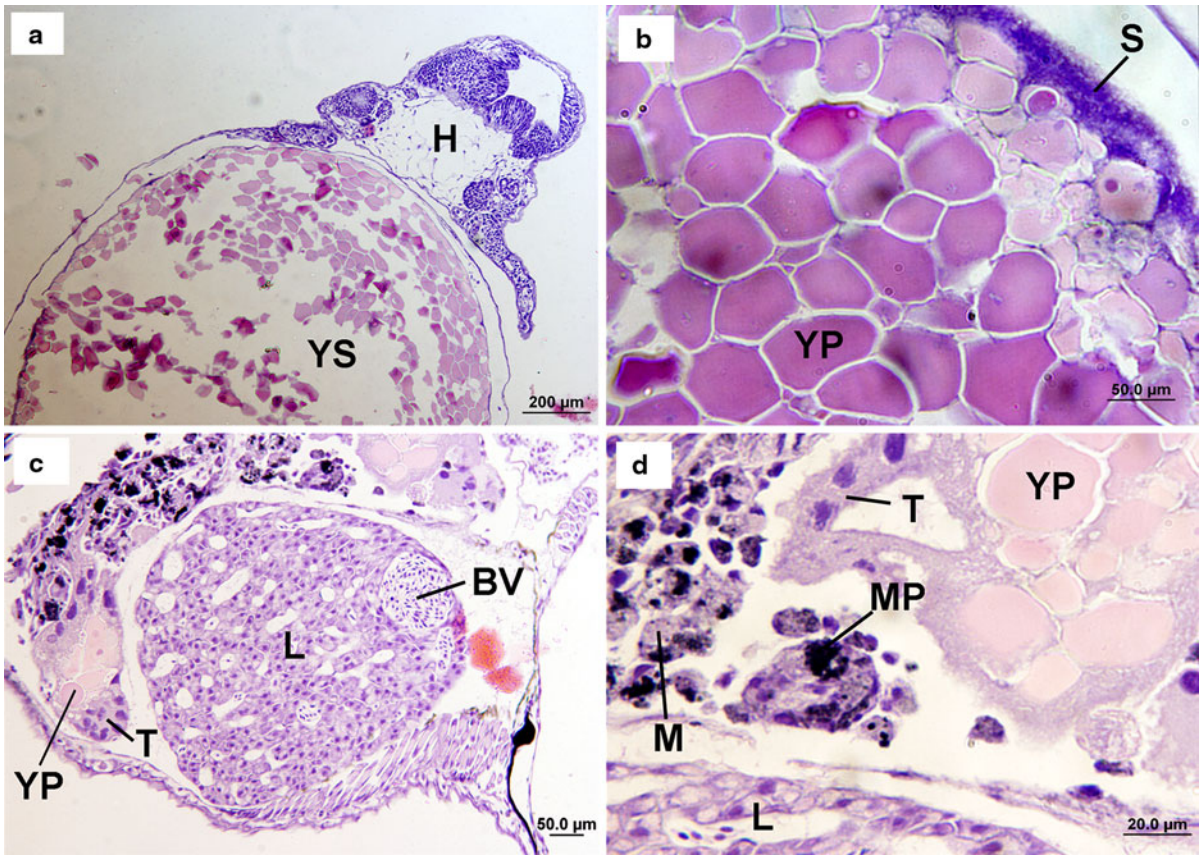



**Fig. 2** Histological sections of the yolk sac in butter catfish. **a** Transversal longitudinal section in a 1-dph larva showing a large eosinophilic yolk sac posterior to the head (the absence of yolk platelets in the yolk sac corresponds to an artefact originated during tissue sectioning). **b** Yolk platelets surrounded by the yolk sac syncytium; note that yolk differing in size as they are close to the epithelial layer surrounding the yolk sac. **c** General view of the abdominal region in a 4-dph larva showing the development of the liver devoid of fat deposits, blood vessel, presence of yolk remnants and tooth. **d** Yolk platelets surrounded by macrophage-like cells and melanin pigment granules connected to venous circulation (age: 4 dph). *Abbreviations*: *BV* blood vessel, *H* head, *L* liver, *M* macrophage-like cell, *MP* melanin pigment, *S* syncytium, *T* tooth, *YP* yolk platelet, *YS* yolk sac. Staining: haematoxylin–eosin

oeseophagus grew in length, and two layers of circular and longitudinal muscular fibres were visible (Fig. 4c). 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. 4d). Between 9 and 11 dph, the oesophagus elongated, and the epithelium lining this region of the digestive tract became columnar and completely covered by mucous cells (Fig. 4e). Under the oesophageal epithelium, a thin layer of connective tissue and striated musculature was detected, flattening and eventually disappearing in the transition from the oesophagus to the stomach (cardiac region) (Fig. 4d). 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 (Fig. 4e) in the posterior region of the oesophagus, which connected it with the glandular stomach.

*Stomach*

The stomach in butter catfish can be divided in two different regions, the non-glandular and glandular stomach, according to their histological organization, developmental timing and functions. Between 6 and 7 dph, the non-glandular stomach started to differentiate

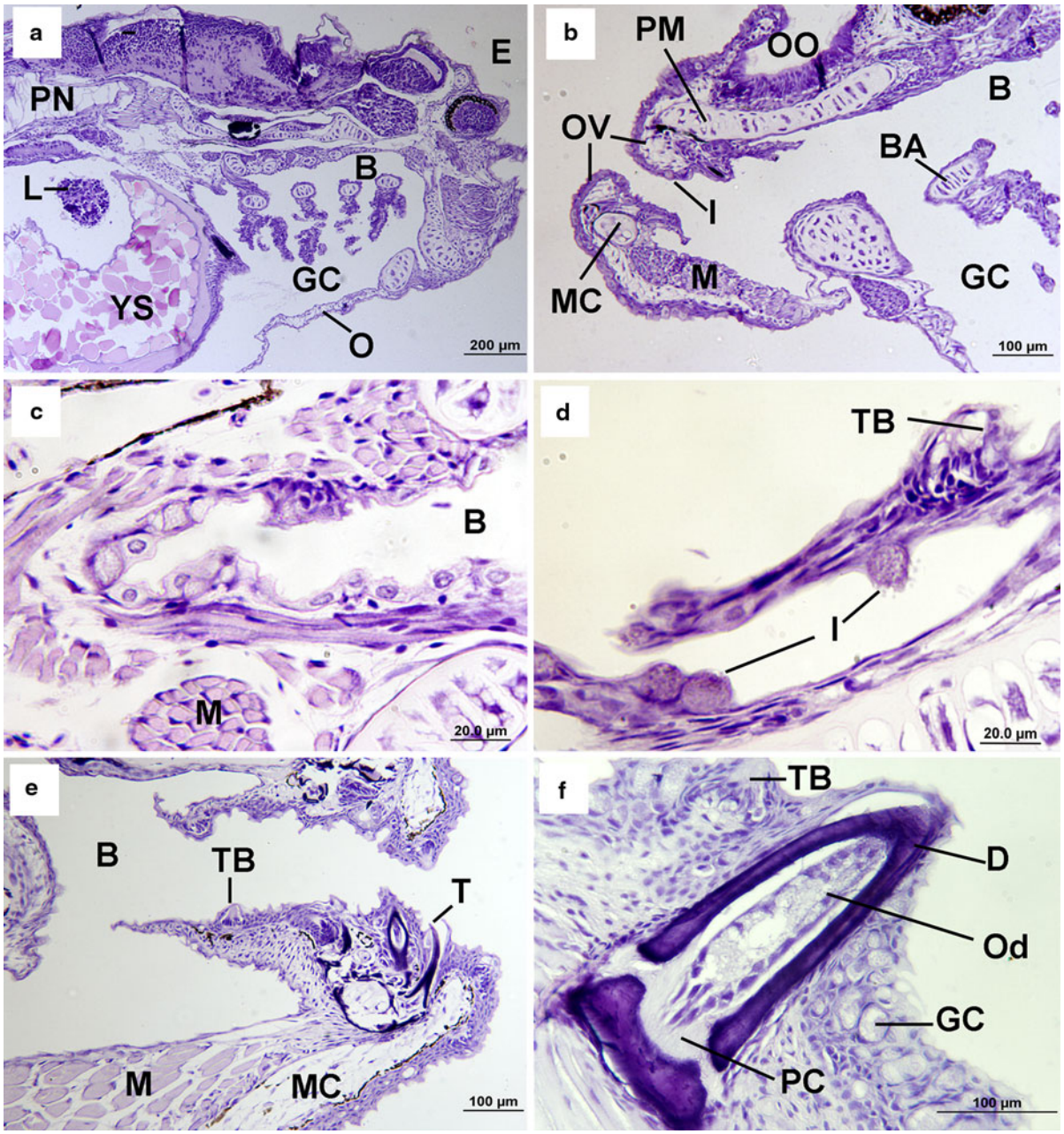



**Fig. 3** Longitudinal histological sections of the buccopharynx of butter catfish at different stages of development. **a** General view of a 1-dph larva showing the buccopharynx, oral cavity, goblet cell, eye, as well as the liver in differentiation, pronephros and a prominent yolk sac. **b** Buccopharynx in a 2-dph larva at the onset of exogenous feeding. **c** Detail of the epithelium of the posterior region of the buccopharynx and smooth musculature. **d** Taste bud and ionocytes lining the epithelium of the buccopharynx close to the opercular cavity. **e** General view of the anterior region of the buccopharynx with two prominent oral valves and numerous teeth protruding into the mouth from the Meckel's cartilage. **f** Detail of a canine-like teeth before protruding into the buccopharyngeal cavity in a 7-dph larva. *Abbreviations*: *B* buccopharynx, *BA* branchial arch, *D* dentine, *E* eye, *G* gill cavity, *GC* goblet cell, *I* ionocyte, *L* liver, *M* smooth musculature fibres, *MC* Meckel's cartilage, *O* oral cavity, *OD* odontoblasts, *OO* olfactory organ, *OV* oral valve, *PM* premaxilla, *PC* pulp cavity, *T* canine-like tooth, *TB* taste bud, *YS* yolk sac. Staining: haematoxylin–eosin

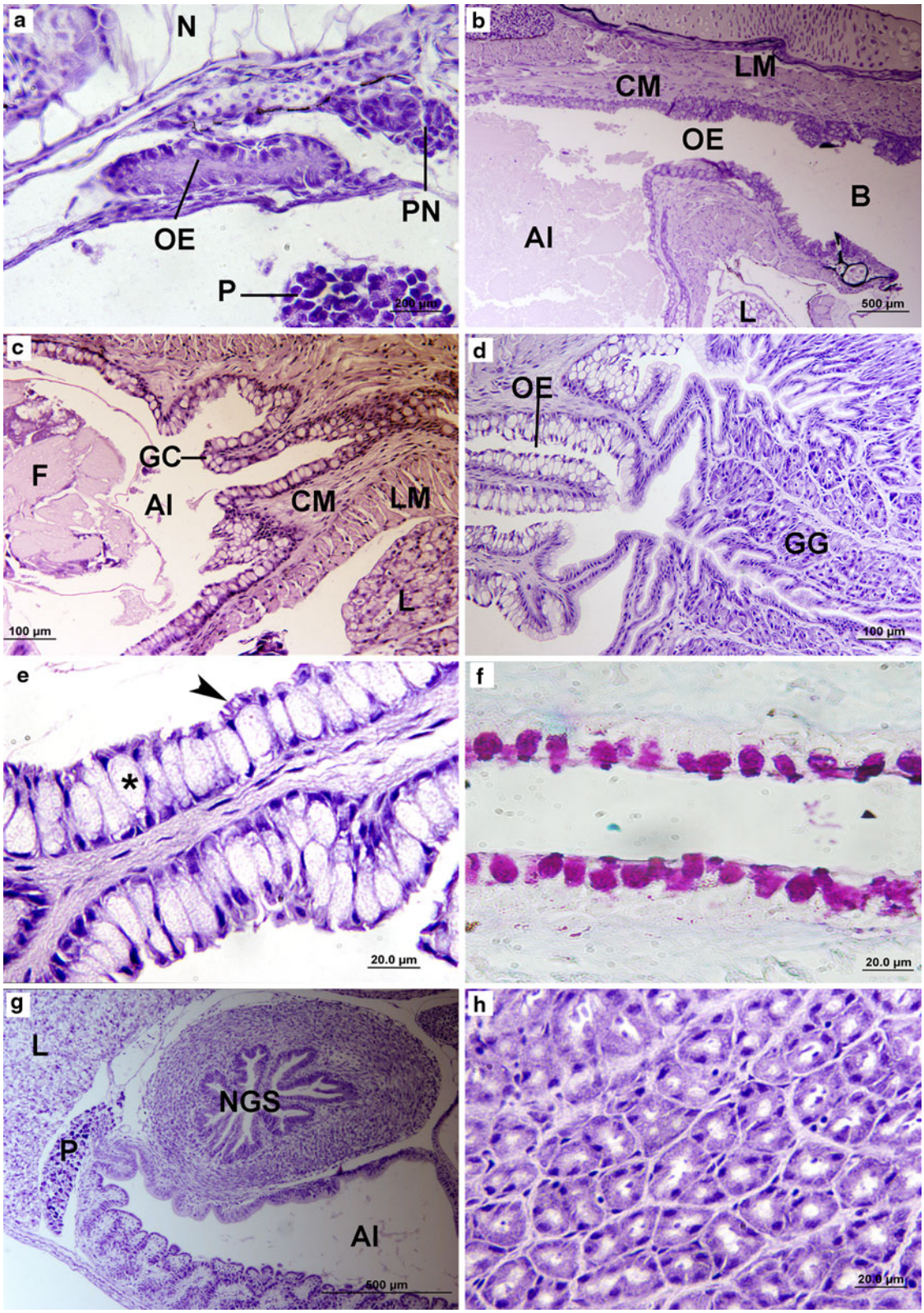
close to the anterior intestine with prominent longitudinal folds lined by a simple short columnar ciliated epithelium surrounded by a thick *tunica muscularis* and a thin serosa (Fig. 5d). At 8 dph, the stomach grew in size, and two different regions were clearly distinguishable, the cardiac region, where gastric glands started to differentiate as clusters of cubic cells (Fig. 4d), and the fundic region where the non-glandular stomach was already differentiated. Between 9 and 11 dph, the glandular stomach greatly developed occupying most part of the abdominal cavity, and abundant gastric tubular glands were arranged along numerous longitudinal folds (Fig. 4h). These tubular glands were composed of a single type of secretory cells devoid of microvilli on their apical border and lining their base with a simple cubic epithelium. The wall of the glandular stomach was composed of mucosa, lamina propria-submucosa, thin muscularis and serosa layers. The pyloric sphincter appeared as an epithelial fold that separated the stomach from the anterior intestine. From 11 to 12 dph to the end of the study, the stomach increased in size and complexity by means of an increase in the size and number of mucosal folds in both cardiac and fundic regions, as well as by an increase in gastric glands in the *cardias* and the thickness of the *tunica muscularis* in the pyloric region, but no relevant histological modifications were observed.

*Intestine*

The intestine was the longest portion of the digestive tract and one of the first organs of the digestive system to differentiate in butter catfish larvae. At 1 dph, it appeared as a straight tube lined by a columnar epithelium bordered by a layer of eosinophilic microvilli at the apical surface. The intestinal absorptive cells or enterocytes were arranged in a single layer and contained medium to basally located nuclei. The intestinal mucosa was surrounded by a thin musculature that was composed of two muscular tissue layers: one circular internal and another longitudinal external separated by a very thin connective tissue layer. At 2 dph, the posterior region of the intestine bent 90°, and the intestinal valve appeared as a constriction of the intestinal mucosa dividing the intestine in two regions, the pre-valvular (anterior) and post-valvular (posterior) intestine (Fig. 5a). The anterior and posterior intestine differed in the size of transversal mucosal folds that were larger in the anterior than in the posterior intestine (Fig. 5c). The rectum was also distinguishable at this age as a short intestinal flattened segment of the intestine devoid of mucosal folds. At this level, the urinary bladder emerged outside the digestive tract posterior to the anus (Fig. 5b). Between 3 and 4 dph, the first goblet cells were detected in the intestinal mucosa and their number tended to increase along larval development. In general, goblet cells were more abundant in the posterior than in the anterior intestine. At 4 dph, the intestine elongated, and small apical lipid droplets ($70.4 \pm 16.4\ \mu m^3$) were detected in scattered enterocytes of the posterior intestine (Fig. 5e), whereas between 5 and 6 dph, a large increase in lipid accumulation was observed in both regions of the intestine, although intra- and inter-cellular fat deposits were more prominent in the posterior intestine and mainly characterized by large vacuoles ($502.7 \pm 43.9\ \mu m^3$) instead of small lipid droplets, resulting in the displacement of nuclei to a basal position (Fig. 5f, g). At 7 dph, the intestine coiled, whereas between 9 and 10 dph, the level of lipid inclusions progressively decreased, and lipids were mainly found in the intestinal mucosa as small apical droplets within enterocytes (Fig. 5h). At 12 dph, the level of lipid deposits in the intestinal mucosa was the lowest ($30.7 \pm 6.1\ \mu m^3$) in comparison with younger ages and remained low till the end of the study (Fig. 5i). No relevant modifications were observed regarding the histological organization of the intestine until the end of the study, with the exception of increase in the length of the intestine, as well as the size and number of mucosal folds.

*Liver and pancreas*

At 1 dph, a well-vascularized lobular liver was present in the abdominal cavity extending from the cardiac region to the head kidney (pronephros). Histologically, this accessory digestive gland appeared as a mass of polyhedral hepatocytes that were loosely organized around a central vein and hepatic sinusoids. Sinusoids were covered by endothelial cells with flattened nucleus and contained mainly erythrocytes in their lumen. During the first 2 days, hepatocytes in butter catfish larvae showed a basophilic homogeneous cytoplasm and a central nucleus with a prominent nucleolus (Fig. 6a), whereas between 3 and 4 dph, the granulation and vacuolization of the cytoplasm due to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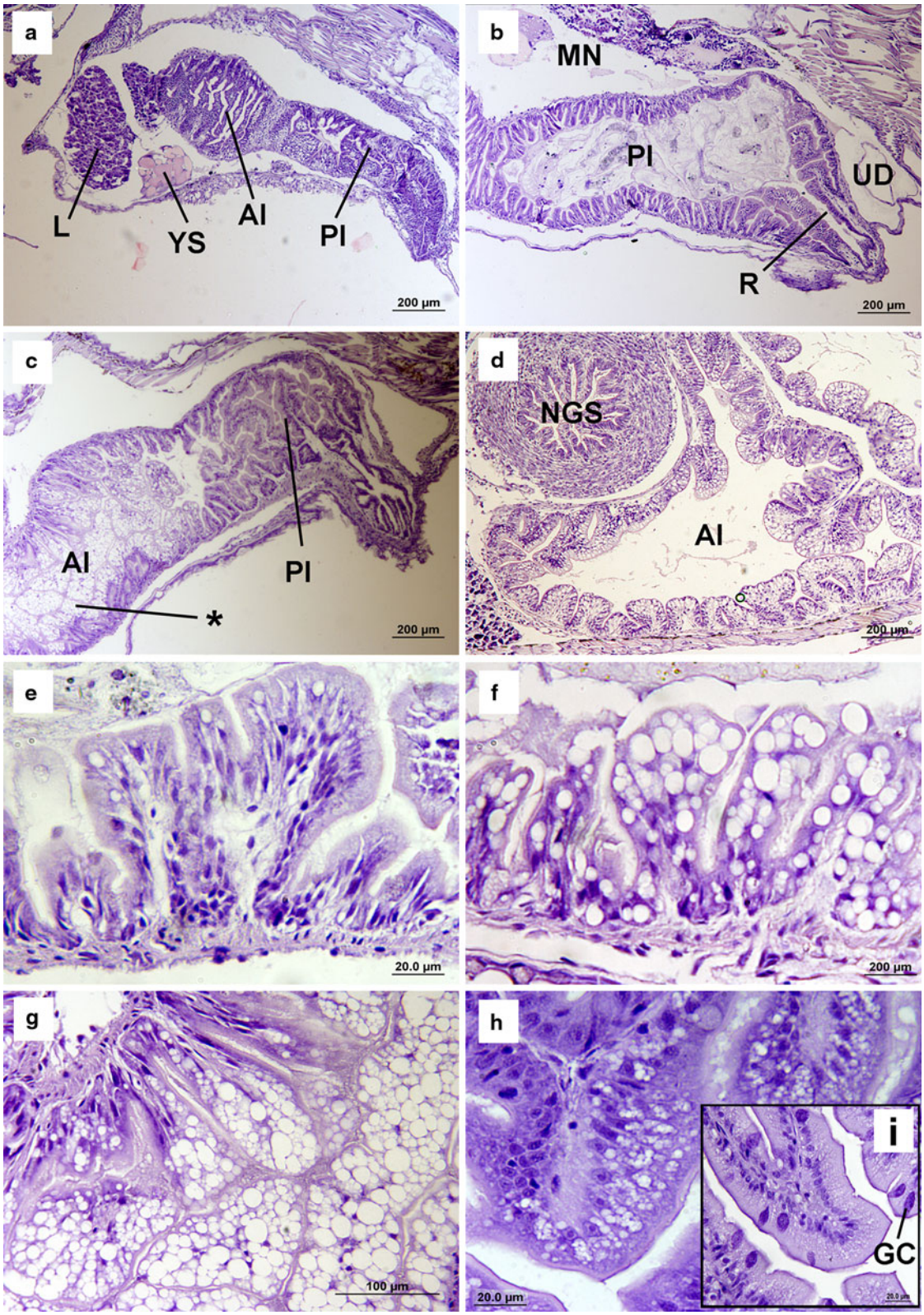
◀ **Fig. 4** Histological sections of the oesophagus and stomach of butter catfish at different stages of development. **a** Detail of the developing oesophagus in a 1-dph larva. **b** Connection of the buccopharynx with the anterior intestine by means of a short oesophagus deprived of folding in a 4-dph larva. **c** Detail of the oesophageal mucosa in a 7-dph larva. Note the prominent circular and longitudinal muscular layers, as well as the entire covering of the oesophageal epithelium by goblet cells. **d** General view of the transition between the oesophagus and gastric stomach showing gastric tubular glands arranged along longitudinal folds (age: 11 dph). **e** Detail of the oesophageal epithelium completely lined by goblet cells (age: 13 dph); note the prominent microvilli covering the epithelium (*arrowhead*) and the colloidal content of goblet cells (*asterisk*). **f** Detail of oesophageal mucous cells containing neutral mucosubstances positively stained with periodic acid-Schiff (PAS) in a 13-dph larva. **g** General view of the non-glandular stomach and anterior intestine in a 7-dph larva. **h** Detail of gastric glands of a 12-dph larva. *Abbreviations*: *B* buccopharynx, *AI* anterior intestine, *BN* bone, *F* food particles, *CM* circular muscle fibres, *GC* goblet cell, *GG* gastric gland, *GS* glandular stomach, *L* liver, *LM* longitudinal muscle fibres, *N* notochord, *NGS* non-glandular stomach, *OE* oesophagus, *P* exocrine pancreas, *PN* pronephros, *TB* taste bud. Staining: haematoxylin–eosin with the exception of 4f that was stained with PAS

synthesis and storage of macromolecules (glycogen, proteins and lipids) started to increase (185 ± 45 μm$^3$). Between 5 and 7 dph, hepatocytes presented large lipid vacuoles (1,925 ± 325 μm$^3$) occupying most part of the cytoplasm and displacing the nucleus to the periphery of the cell (Fig. 6b). Concomitantly, with such increase of fat deposits in the liver, mesenteric adipose tissue was found surrounding the pancreas, spleen and posterior intestine (Fig. 6h). The level of hepatic lipid deposits increased until 11 and 12 dph (3,140 ± 425 μm$^3$), whereas after this age, the level of fat deposits decreased (1,527 ± 363 and 256 ± 41 μm$^3$ at 15 and 21 dph, respectively), and glycogen deposits (PAS-positive) were observed in the hepatocyte's cytoplasm (Fig. 6c, d). As the larvae grew, the liver increased in size and occupied most of the anterior part of the abdominal cavity.

At 1 dph, the exocrine pancreas was already differentiated in polyhedral basophilic cells arranged in acini containing round-shaped eosinophilic zymogen granules (Fig. 6e). At 2 dph, pancreatic acinar cells were grouped in rosette patterns around central canals that anastomosed with large pancreatic ducts. The pancreatic duct was lined with a squamous epithelium and opened into the ventral part of the anterior intestine just after the pyloric sphincter. Between 7 and 8 dph, the endocrine pancreas differentiated, and endocrine cells arranged around many capillaries were grouped in a single islet of Langerhans. The most relevant changes in the histological organization of the pancreas from 8 dph to the end of the study were increase in size and number of pancreatic acini, as well as in the number of the islets of Langerhans (Fig. 6g).

## Discussion

Anatomically, the digestive tract in butter catfish was similar to other siluriform species such as the African catfish *Clarias gariepinus* (Verreth et al. 1992), the slender walking catfish *Clarias nieuhofii* (Saelee et al. 2011), the yellow catfish *Pelteobagrus fulvidraco* (Yang et al. 2010) and the silver catfish *Rhamdia quelen* (de Amorim et al. 2009), with the exception of the stomach. Butter catfish is the only described catfish species with a non-glandular stomach characterized by a folded mucosa containing mucous cells, deprived of gastric glands and surrounded by a prominent *tunica muscularis*. The thickening and hypertrophy of the muscular layers surrounding the pyloric region of the stomach can be refereed as the gizzard, and it has triturative and grinding functions (Gisbert et al. 1998; Lazo et al. 2011). Important differences in the time of organ differentiation and development were observed between butter catfish and several catfish species described so far (Verreth et al. 1992; Kozarić et al. 2008; de Amorim et al. 2009; Yang et al. 2010; Saelee et al. 2011). The timing of development of organ and its physiological function is affected by the general life history and reproductive strategy of each species and also by a variety of biotic and abiotic factors, including water quality, mainly temperature, and food availability and composition. Consequently, we have decided to focus this section on the ontogenetic changes in the histomorphological organization of digestive organs and their link to rearing practices that could be of use for improving actual larval rearing procedures in this species, as well as the inter-specific comparison of the histological organization and development with other catfish species. As different studies on several catfish species (Verreth et al. 1992; Kozarić et al. 2008; de Amorim et al. 2009; Yang et al. 2010; Saelee et al. 2011) were conducted at different rearing temperatures, data were compared by means of degree day units, whenever it was possible.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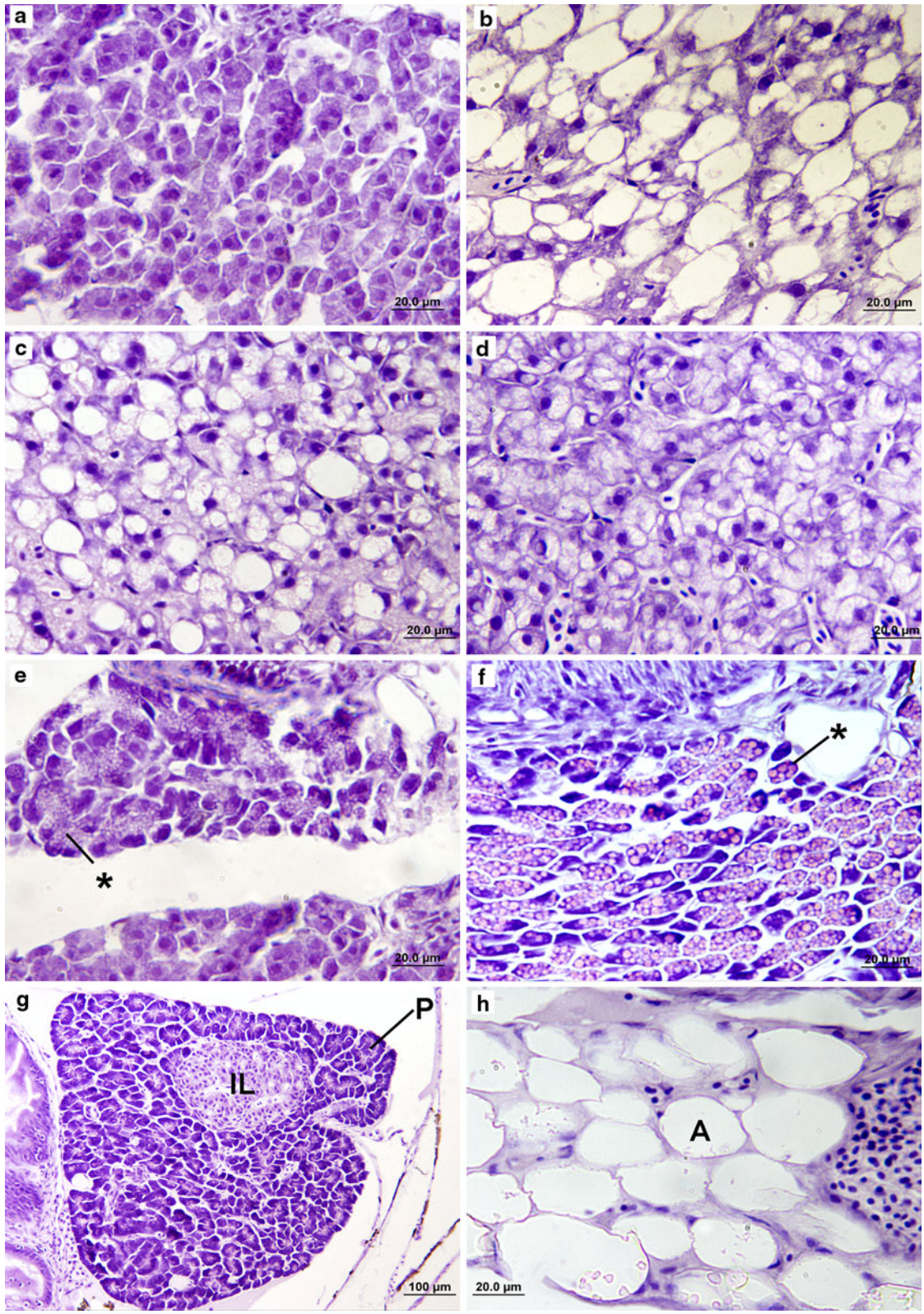
◀ **Fig. 5** Longitudinal histological sections of the intestine of butter catfish (*Ompok bimaculatus*) at different stages of development. **a** General view of the intestine in a 2-dph larva; note the development of the liver, remnants of yolk, anterior intestine and posterior intestine. **b** Detail of the posterior intestine and rectum in a 4-dph larva. **c** General view of the intestine in a 6-dph larva showing the accumulation of fat deposits (*asterisk*) in the anterior intestine and the absence of them in the posterior intestine. **d** Detail of the anterior intestine with lipid deposits in enterocytes in a 7-dph larva. **e–i** Changes in lipid deposition in intestinal villi along ontogeny (**e**, 3 dph; **f**, 4 dph; **g**, 6; **h**, 10 dph; **i**, 12 dph). *Abbreviations*: *AI* anterior intestine, *GC* goblet cell, *MN* mesonephros, *NGS* non-glandular stomach, *PI* posterior intestine, *R* rectum, *UD* urinary duct. Staining: haematoxylin–eosin

In butter catfish, the mouth opening occurred between 1 and 2 dph (27–54 degree days), similarly to other catfish such as *C. gariepinus* (Verreth et al. 1992), *C. nieuhofii* (Saelee et al. 2011) and *P. fulvidraco* (Yang et al. 2010), with the exception of *R. quelen* (de Amorim et al. 2009) that opened its mouth at 96 degree days (Table 1). In most studied catfish species, with the exception of *R. quelen*, there is a mixed feeding phase during which an overlap of endogenous and exogenous feeding occurs. The length of this period varies among species, ranging from 54 degree days in *O. bimaculatus* to 69 and 80 degree days in *R. quelen* (de Amorim et al. 2009) and *S. glanis* (Kozarić et al. 2008), respectively. A mixed feeding phase generally affects growth, survival and larval development by neutralizing any potential deficit in nutrient provision prior to completion of yolk reserves during the time of transition to exclusively exogenous feeding as well as serves as a temporary reserve of nutrients for the larva to withstand short periods of food deprivation (Treviño et al. 2011). The transition to exogenous food in the presence of yolk reserves generally implies that the alimentary canal is functional, although structural and functional development still continues from the larval to the juvenile and adult forms (Jaeroszewska and Dabrowski 2011). In this sense, to ensure that young larvae can digest, absorb and assimilate nutrients from exogenous food during this period of mixed nutrition, a minimum set of digestive structures and functions must be developed (Segner et al. 1993). In the present study, histological observations suggested that butter catfish larvae aged 2 dph (54 degree days) were able to ingest, digest and assimilate nutrients from exogenous food, as the presence of digested prey items in the lumen of the gut, eosinophilic zymogen granules in the exocrine pancreas and fat deposits in the intestine and liver indicated. This precocial development of the digestive system has been also described in the rest of described catfish species (Verreth et al. 1992; Kozarić et al. 2008; de Amorim et al. 2009; Yang et al. 2010; Saelee et al. 2011), as well as in other freshwater fish species, such as salmonids (Rust 2002), acipenserids (Gisbert et al. 1998; Gisbert and Doroshov 2003; Wegner et al. 2009) and cichlid species (Morrison et al. 2001; Treviño et al. 2011 among others).

The timing of the development of teeth, tongue, taste buds and mucous cells in the buccopharynx of butter catfish was similar to the pattern described in *P. fulvidraco* (Yang et el. 2010) and coincided with the transition of the larva to the exogenous feeding, which might be attributed to the involvement of the above-mentioned structures in the capture, taste and swallowing of preys. The butter catfish larvae prey on small insects, nematodes, annelids, fish scales, fish eggs and other fish larvae (Weliange and Amarasinghe 2007); thus, the abundance of goblet cells in the posterior region of the buccopharynx and oesophagus and their secretions rich in neutral glycoconjugates might serve as a lubricant from protecting the buccopharyngeal and oesophageal mucosa from the abrasion that ingesting these prey could produce (Gisbert et al. 1999). These results were in agreement with those reported in *S. glanis* (Kozaric et al. 2008) and *P. fluvidraco* (Yang et al. 2010). The development of the buccopharyngeal mucosa in the above-mentioned species was coupled with the differentiation of the oesophagus development of longitudinal folds and increase in the number of goblet cells producing neutral glycoconjugates, which in butter catfish occurred between 8 and 11 dph (216–297 degree days). In addition to the above-mentioned role as lubricant, neutral glycoconjugates secreted by oesophageal globets cells could also cooperate in the digestion of food and its transformation into chyme, as well as in the absorption of easily digested substances (Sarasquete et al. 2001).

In the present study, the first lipid vacuoles in the intestinal mucosa were observed at 4 dph (108 degree days), at about 2 days after the onset of exogenous feeding. Similar results were reported in *S. glanis* (Kozaric et al. 2008) and *C. nieuhofii* (Saelee et al. 2011), whereas fat inclusions in enterocytes of *C. gariepinus* (Verreth et al. 1992) and *P. fulvidraco*


a
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◀ **Fig. 6** Histological sections of the accessory digestive organs of butter catfish at different stages of development. **a** Liver in a 2-dph larvae showing polyhedral hepatocytes arranged along hepatic sinusoids with no signs of lipid deposition. **b** Liver in a 6-dph larvae showing large lipid deposits within hepatocytes (unstained deposits within hepatocytes corresponded to lipids dissolved during the embedding process of the larva in paraffin); note the displacement of the nuclei to the periphery of the cell. **c**, **d** Fat deposition within hepatocytes at 13 and 21 dph, respectively; note the reduction of fat deposits between both ages with regard to 6 dph (**b**). **e** Exocrine pancreas in a 2-dph larvae showing polyhedral basophilic pancreocytes arranged in acini containing zymogen granules (*asterisk*). **f** Detail of the exocrine larvae of a 6-dph larvae; note the increase in size and acidophilia of zymogen granules (*asterisk*) in pancreocytes. **g** General view of the exocrine and endocrine pancreas (islet of Langerhans). **h** Detail of adipose tissue (mesenteric fat deposits) surrounding the pancreas (age: 30 dph). *Abbreviations*: *A* adipose tissue, *P* exocrine pancreas, *IL* endocrine pancreas (islet of Langerhans). Staining: haematoxylin–eosin

(Yang et al. 2010) were detected within the first hours after the onset of exogenous feeding. In fish larvae, intestinal lipid inclusions are considered a temporary storage form of absorbed re-esterified fatty acids in cases when the rate of lipid absorption exceeds the rate of lipoprotein synthesis (Sheridan 1988) or because of an inability to metabolize lipids (Kjørsvik et al. 1991). Under normal conditions, the rapid development of the intestinal enterocytes during larval growth is combined with increasingly effective lipoprotein synthesis, which is accompanied by a considerable decrease in the number of large lipid vacuoles in the enterocytes, as well as an important increase in the number of small lipid particles in the intercellular spaces (Deplano et al. 1991; Sarasquete et al. 1995; Gisbert et al. 2008). In contrast to the other catfish species considered in this study and other teleosts (Lazo et al. 2011), fat deposits in butter catfish larvae were more abundant in the posterior than in the anterior intestinal segments. Similarly to the results observed in butter catfish, some authors have reported that lipid digestion and absorption continue in the posterior region of the intestine (Zambonino-Infante et al. 2008). In this study, no relevant differences were observed in the histological organization of the anterior and posterior regions of the intestine, with the exception of the number and size of mucosal folds, which are longer and deeper, and the degree of fat accumulation within enterocytes. During butter catfish larval development, changes in fat deposition were observed in the intestinal mucosa. These changes were characterized by an important increase in lipid deposition between 5 and 6 dph, during the peak of the *Artemia* feeding phase, whereas fat deposits progressively decreased when larvae were progressively transferred from *Artemia* nauplii to natural zooplankton, mainly copepods. As Øie et al. (2011) recently reviewed, copepods have generally lower total lipid and neutral lipid contents as compared with traditionally cultured live prey like *Artemia*, but have a higher proportion of easily digestible phospholipids. Thus, changes in the lipid accumulation in the intestinal mucosa of butter catfish seemed to be due to the nature of the dietary lipids, since phospholipids are generally more easily digested, absorbed and transported from enterocytes to other tissues than neutral lipids (Gisbert et al. 2005). Feeding this species with *Artemia* for long periods might be not recommendable, since the high levels of fat and neutral lipid contents of this prey might result in a large intestinal accumulation of fat, which could ultimately lead to intestinal steatosis, impairing the integrity of the intestinal mucosa and affecting the proper absorbance of nutrients (Mobin et al. 2000).

In the present study, lipid accumulation appeared in the liver between 3 to 4 dph and increased progressively until 11 to 12 dph, when the level of fat deposits decreased and glycogen deposits were observed in hepatocytes's cytoplasm. Similar results have been observed in *S. glanis* (Kozarić et al. 2008) and *P. fulvidraco* (Yang et al. 2010), which tended to store glycogen and lipid reserves in the liver as their digestive system gradually developed and the pancreatic and intestinal functions became established (Zambonino-Infante et al. 2008).

In butter catfish, gastric glands started to differentiate at 8 dph, and the stomach was completely differentiated with abundant gastric tubular glands arranged along numerous longitudinal folds by 11–12 dph (297–324 degree days). The appearance of gastric glands normally indicates the formation of a functional stomach (Stroband and Kroon 1981), which is also a histological criterion to differentiate larvae from juveniles (Tanaka 1971; Sarasquete et al. 1995). In addition, the presence of mucous cells in the epithelium lining the stomach lumen, producing and secreting neutral glycoconjugates confirmed that between

**Table 1** Comparison of major developmental events of the digestive system ontogeny in different siluriform species

| Developmental events | *O. bimaculatus*[1] *Siluridae* | | *S. glanis*[2] *Siluridae* | | *P. fulvidraco*[3] *Bagridae* | | *C. nieuhofii*[4] *Claridae* | | *C. gariepinus*[5] *Claridae* | | *R. quelen*[6] *Heptapteridae* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ADD | DPH | ADD | DPH | ADD | DPH | ADD | DPH | ADD | DPH | ADD | DPH |
| Appearance of intestine | 27 | 1 | 69 | 3 | 24 | 1 | 53 | 2 | 27.5 | 1 | NA | NA |
| Appearance of incipient liver and pancreas | 27 | 1 | 69–115 | 3–5 | 48 | 2 | 40–53 | 1.5–2 | 27.5 | 1 | NA | NA |
| Appearance of zymogen granules in the pancreas | 27 | 1 | 69–115 | 3–5 | 96 | 4 | NA | NA | 55 | 2 | NA | NA |
| Mouth opening | 27–54 | 1–2 | NA | NA | 24–48 | 1–2 | 53 | 2 | 55 | 2 | 96 | 4 |
| Onset of exogenous feeding | 54 | 2 | 92 | 4 | 72 | 3 | 106 | 4 | 55 | 2 | NA | NA |
| Intestine differentiation | 54 | 2 | 92–138 | 4–6 | 72 | 3 | 106 | 4 | 27 | 1 | 72 | 3 |
| Oesophagus differentiation | 54–81 | 2–3 | 92–138 | 4–6 | 96 | 4 | 106 | 2 | NA | NA | NA | NA |
| Granulation and vacuolization of hepatocyte cytoplasm | 81–108 | 3–4 | 115–161 | 5–7 | 264–312 | 11–13 | 53–265 | 2–10 | NA | NA | NA | NA |
| Appearance of lipid droplets in the intestine | 108 | 4 | 207 | 9 | 72 | 3 | 106 | 4 | 82.5 | 3 | NA | NA |
| Yolk sac exhaustion | 135 | 5 | 161 | 7 | NA | NA | 185 | 7 | 60 | 2 | 96 | 4 |
| Gastric glands appearance | 216 | 8 | 115–161 | 5–7 | 72 | 3 | 106 | 4 | 110–138 | 4–5 | NA | NA |
| Morphologically complete digestive system | 324 | 12 | 161 | 7 | 600 | 25 | 133 | 5 | 193 | 7 | NA | NA |
| Average rearing temperature | 27.0 °C | | 23.5 °C[a] | | 24.0 °C | | 26.5 °C | | 27.5 °C | | 24.0 °C | |

For comparative purposes, larval development is shown in accumulated degree days (ADD) and days post-hatching (DPH)

[1] Present study; [2] Kozaric et al. (2008); [3] Yang et al. (2010); [4] Saelee et al. (2011); [5] Verreth et al. (1992); [6] de Amorim et al. (2009)

*NA* data not available

[a] Personal communication

11 and 12 dph, the stomach was probably functional in this species, since neutral mucosubstances may protect the stomach from autodigestion processes caused by HCl and enzymes produced by gastric glands (Sarasquete et al. 2001; Chen et al. 2006). With regard to the other catfish considered in this study, the stomach developed earlier in *P. fulvidraco* (Yang et al. 2010), followed by *C. nieuhofii* (Saelee et al. 2011), *C. gariepinus* (Verreth et al. 1992) and *S. glanis* (Kozaric et al. 2008), the butter being catfish the species in which the stomach developed the last.

Although gastric glands developed soon after hatching in most of described catfish species with the exception of butter catfish, Yang et al. (2010) reported that the presence of pinocytotic processes in the intestinal mucosa, coupled with differentiated gastric glands for a long period of time, and the gradual replacement of protein pinocytotic absorption and intracellular digestion by extracellular digestion indicated that gastric glands were not completely functional until later stages of development. In this sense, several authors have shown that the development of a functional stomach associated with the production of HCl and pepsin by gastric glands is a crucial event for enabling young fish to digest compound diets (Zambonino-Infante et al. 2008). Although *C. gariepinus* larvae are able to be reared on microdiets from the onset of exogenous feeding, their growth rates were not comparable to those fed live food until their stomach became functional (Verreth and van Tongeren 1989; Verreth et al. 1992). Therefore, the larval period in terms of nutrition ends with the completion of a functional stomach (Segner et al. 1993), and consequently, the stomach differentiation could be considered as a decisive event in the nutritional physiology of larvae and could be used as an external marker for the start of weaning (Verreth et al. 1992; Senger et al. 1993). Hence, it might be recommended to wean butter catfish larvae into microdiets when they have a morphologically complete digestive system at 12 dph when the larval period ends from a nutritional physiology point of view characterized by a putative transition from alkaline to acid digestion. Further research must be conducted on assessing the functionality of the digestive system, considering the production of pancreatic, stomach and intestinal enzymes in order to confirm present results and to enlarge the knowledge on the digestive physiology of this species under different nutritional conditions.

## Conclusions

The development of butter catfish digestive system was accomplished within the first 12 days after hatching (324 degree days), when larvae showed a histomorphological well-differentiated digestive system composed of long oesophagus covered with PAS-positive mucus producing goblet cells, a prominent stomach divided into a non-glandular and a glandular stomach, a long and folded intestine in which lipids were prominently absorbed in the posterior intestinal segment, and functional accessory glands (liver and pancreas). Butter catfish larvae displayed a similar pattern of ontogenetic developmental pattern of the digestive system similar to other catfish species and other teleosts, although their timing of development was much shorter in this group of fish, indicating the fast growing nature of catfish. Our findings on the development of the digestive system in butter catfish could lead to a better understanding of the digestive physiology in butter catfish larvae, which might be useful for improving the current larval rearing techniques in this species, a promising catfish species for freshwater aquaculture diversification in the subcontinent.

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