

DEVELOPMENTS OF DIGESTIVE TRACT IN LARVAE OF CLIMBING PERCH, *Anabas testudineus* (Bloch)

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ABSTRACT

Climbing perch, *Anabas testudineus* (Bloch) is a potential species for aquaculture in Kalimantan, Indonesia and belongs to Anabantidae family. The development of its digestive tract was evaluated on larvae reared under culture conditions of 28°C-30°C, from hatching to 30 days after hatching using histological and morphological methods. The larvae were kept in six 100-L tanks. They were fed with rotifers and microalgae from day 2nd after hatching to day 10th; *Artemia* nauplii from day 7th to day 15th; *Artemia* meta-nauplii from day 15th to day 20th; and *Tubifex* worm from day 20th onwards. The development of digestive tract in climbing perch followed the general pattern described for other species. Shortly after hatching, its digestive system was found to be consisted of an undifferentiated straight tube laying dorsally to the yolk sac. At first feeding (day 2nd), both mouth and anus had opened and the yolk sac was partially absorbed. On day 3, the digestive tract was fully differentiated into buccopharynx, esophagus, intestine and rectum. The two pyloric caeca appeared on day 25th after hatching, indicating the transition from larval to juvenile stage and acquisition of an adult type of digestion.

KEYWORDS: digestive tract, larvae, development, climbing perch

INTRODUCTION

Larval nutritional studies have been receiving much attention because an understanding of changes associated with the processes of food ingestion, digestion, and assimilation are the initial steps in determining the ability of larvae to utilize a given diet (Martinez *et al.*, 1999). The high rates of mortality reported during small larval fish culture have been blamed to inadequate larval nutrition. A change in the source of nutrition will occur during larval development. In the first day, its endogenous is sourced from the yolk sac and oil glob-

ule. Once the endogenous reserves are all absorbed, food must be obtained from an exogenous source. At the time of the first feeding, the larval digestive tract is not fully developed (Micale *et al.*, 2006) so that small larval rearing is based primarily on the use of live prey during early development of larvae from two to several weeks.

A detail understanding of the development of the digestive tract of fish larvae may contribute to the increase of the larval rearing success. This gut ontogeny study is an initial step in identifying and implementing new feed

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alternatives for small fish larvae. Together with digestive enzymatic study, it can determine the best time to initiate early weaning of the larvae. This practice may result in substantial cost savings in live food production. Several commercially valuable species have been studied, including seabass (Walford & Lam, 1993), gilthead seabream (Sarasquete *et al.*, 1995), spotted sand bass (Pena *et al.*, 2003), California halibut (Gisbert *et al.*, 2004), yellow-tail kingfish (Chen *et al.*, 2006), goldlined seabream (Ibrahim *et al.*, 2006), common pandora (Micale *et al.*, 2006), and percula clownfish (Onal *et al.*, 2008). However, little information is available regarding the digestive tract development in larvae of tropical freshwater fish species.

The climbing perch (*A. testudineus*) is a member of anabantoid fish and has a potential to be cultured in Kalimantan. It has been successfully reared under controlled laboratory conditions until adult stage (Phuong *et al.*, 2001; Widodo *et al.*, 2007), but high larval mortality has been reported (Trieu & Long, 2001; Morioka *et al.*, 2009). Therefore, the objective of this study was to describe the ontogenetic development of the digestive tract of climbing perch larvae under culture conditions, for the purpose of improving feeding strategies during this developmental stage.

MATERIAL AND METHODS

Larvae used in the present study were obtained from an artificial spawning of *A. testudineus* broodstock (3 females and 6 males) of climbing perch in the laboratory of Aquaculture Department, Bogor Agricultural University. Newly hatched larvae were placed in three 100-L tanks with water temperature of 28°C - 30°C. Larvae were fed for the first time at day 2nd after hatching until day 10th after hatching with rotifers (*Brachionus calyciflorus*) and microalgae. From day 7th to 15th, the fish were fed with newly *Artemia* nauplii, and then continued fed with *Artemia* meta-nauplii until day 20th after hatching. *Tubifex* worms were used as live preys from day 20th to day 30th post hatching.

A hundred larval samples were taken from the rearing tanks on day 0-5th, 8th, 12th, 16th, 20th, 25th, and 30th after hatching to measure their total length and mouth size using a microscope connected to a digital camera. The

samples (10 larvae) were fixed using Bouin's solution before further analysis. For each sampling day, a fixed larvae sample was dehydrated with a graded series of ethanol (70% - 100%). The larvae were embedded in paraffin. Sagittal sections (3-5 µm thick) were obtained with a microtome and stained with haematoxylin-eosin for general acidophilic and basophilic histological features. Slides were also stained by periodic acid-Schiff (PAS) for neutral mucosubstances. Observations using light microscope were performed using different magnifications to describe the development of the digestive tract.

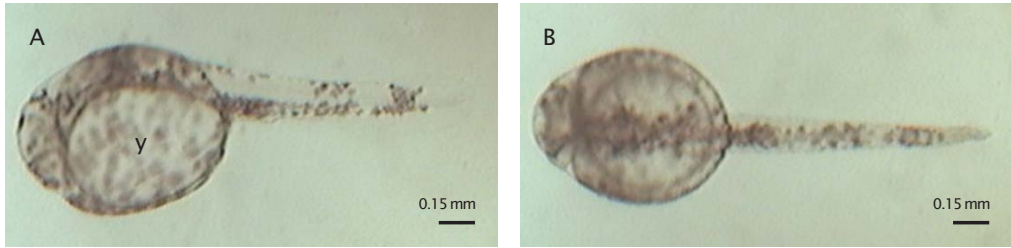
RESULT AND DISCUSSION

Results

Morphology

At hatching (day 0, H0), the total length of yolk sac larvae was 2.12 ± 0.16 mm ($n = 100$) (Fig. 1) and reached 17.64 ± 3.79 mm ($n = 100$) on day 30th. At H0, the alimentary canal appeared as a straight tube lying dorsally to the yolk sac. The mouth and anus were closed. At day 1st to day 2nd after hatching, yolk-sac absorption was very fast and the digestive system of climbing perch underwent further differentiation and the rudimentary alimentary canal became segmented into four different regions: the buccopharynx, esophagus, intestine, and rectum. All of the larvae opened mouths about 34-36h after hatching, with the mouths measuring 0.38 ± 0.03 mm in height. At day 2nd after hatching (H2) with total length 3.03 ± 0.30 mm, $n = 100$, the mouth had opened and exogenous feeding began. At the same day, the eyes were pigmented. Growth of climbing perch larvae followed an exponential curve (Fig. 2).

From day 2nd after hatching the swim bladder started to be inflated. At day 5th after the first feeding, the yolk sac was almost completely reabsorbed. At day 12th after hatching with total length of 5.46 ± 0.89 mm, larvae started to change its morphology to be similar to adult type and metamorphosis processes were completed around day 25th after hatching for most of the larvae. Moreover, two pyloric caeca appeared at day 25th after hatching, indicating the transition from larval to juvenile stage and acquisition of an adult type of digestion.



A. ventrally view; B. Dorsally view; y = yolk; 100X

Figure 1. Newly hatched larva *Anabas testudineus* with average total length 2.12 ± 0.16 mm, n=100

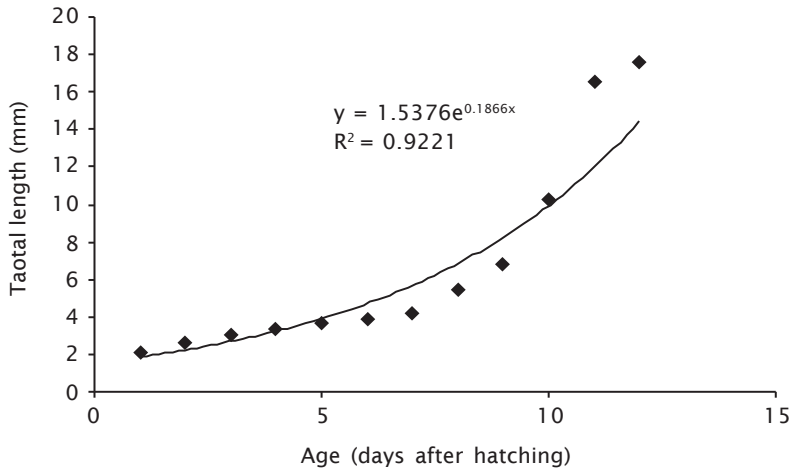


Figure 2. Average total length (mm) of *A. testudineus* larvae. The arrow indicates the time of first feeding at day 2 after hatching

Histology

- Buccopharynx

Between day 1st and 2nd after hatching, the mouth opened. The buccopharyngeal epithelium was composed of a single layer of squamous cells. Chondrocytes of the rudimentary gill arches were visible under the epithelium of the posterior buccopharynx (Fig. 3). At day 12th after hatching the goblet cells appeared PAS positive (Fig.4B). Oral and tongue taste buds as well as buccal goblet cells became evident among epithelial cells from day 25th after hatching (Fig. 4C). Taste buds and goblet cells became more numerous as larvae grew.

- Oesophagus and stomach

At day 2nd after hatching, the buccopharynx channeled with the anterior intestine

through a short oesophagus with a rather narrow lumen (Fig. 4A). At day 16th after hatching, the stomach exhibited a pouched shape (Fig. 5A) and it began well developed at day 20th after hatching (Fig. 5B). At day 30th (total length 17.64 ± 3.79 mm), two pyloric caeca were also observed.

- Intestine

With the beginning of exogenous feeding, the lumen of the anterior intestine dilated and small. At day 12th after hatching mucosal folding appeared well developed in the anterior and posterior intestines (Fig. 6A). At day 30th after hatching, mucosal folding of the anterior and posterior intestines appeared very prominent (Fig. 6B). PAS positive goblet cells appeared distributed between enterocytes of both anterior and posterior intestines from day 30th after hatching (Fig. 6C, D).

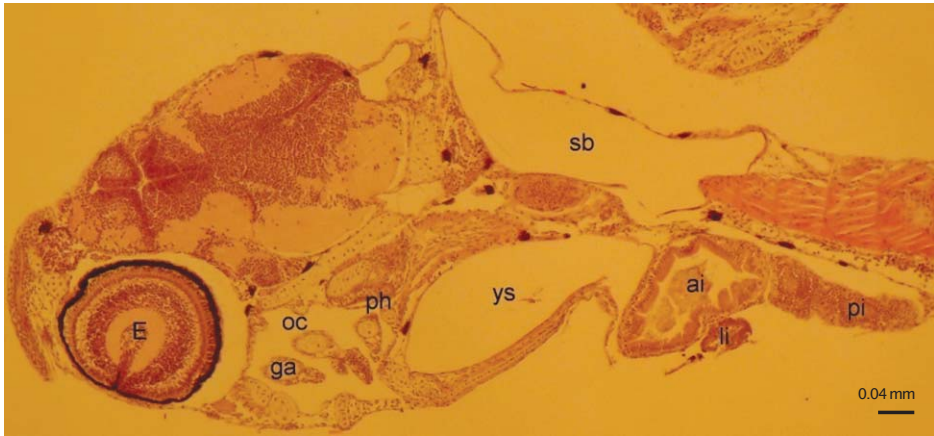


Figure 3. Sagittal sections of the gastro-intestinal tract of climbing perch larvae at day 2 after hatching showing differentiation of oral cavity (oc), pharynx (ph), anterior intestine (ai) and posterior intestine (pi), HE, 100X; yolk sac (ys), eye (E), gill arch (ga), liver (li), swim bladder (sb)

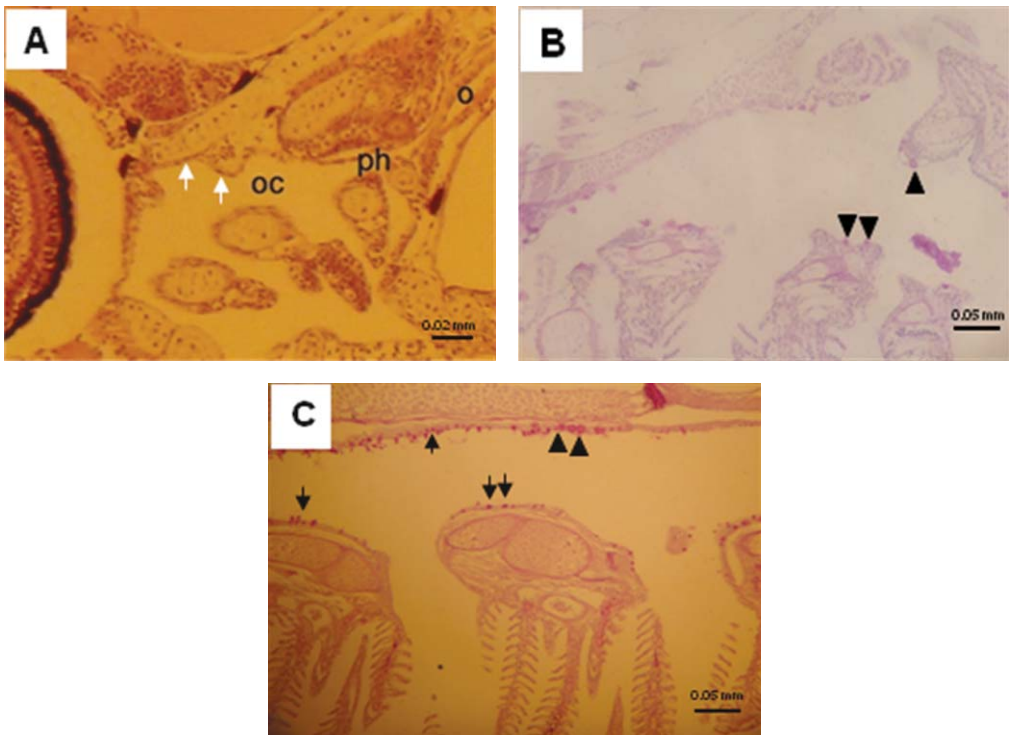


Figure 4. (A) Detail of pharyngeal epithelium at day 2 after hatching of climbing perch larvae (white arrows); HE, 400X. (B) Detail of pharyngeal epithelium at day 12 after hatching larvae showing functional goblet cell (arrowheads); PAS, 200X. (C) Detail of pharyngeal epithelium at day 25 after hatching larvae showing goblet cells (arrowheads) and taste buds (arrows); PAS, 200X; oral cavity (oc), gill arch (ga), pharynx (ph), oesophagus (o)

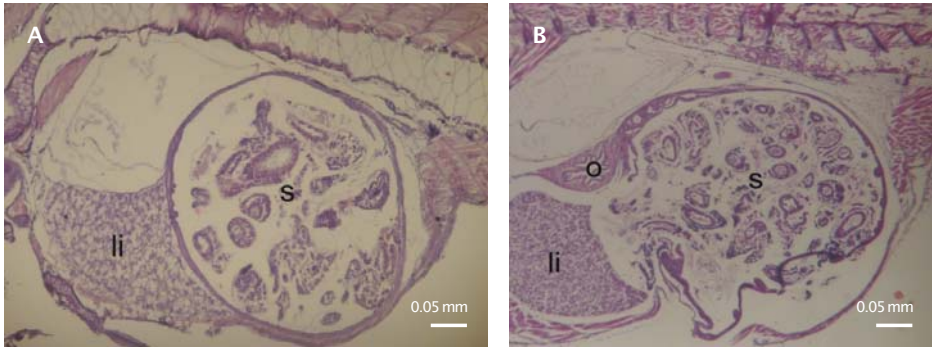


Figure 5. Sagittal sections of stomach of climbing perch larvae at (A) day 16 after hatching. (B) day 20 after hatching showing well development; HE, 200X; stomach (s), liver (li), oesophagus (o)

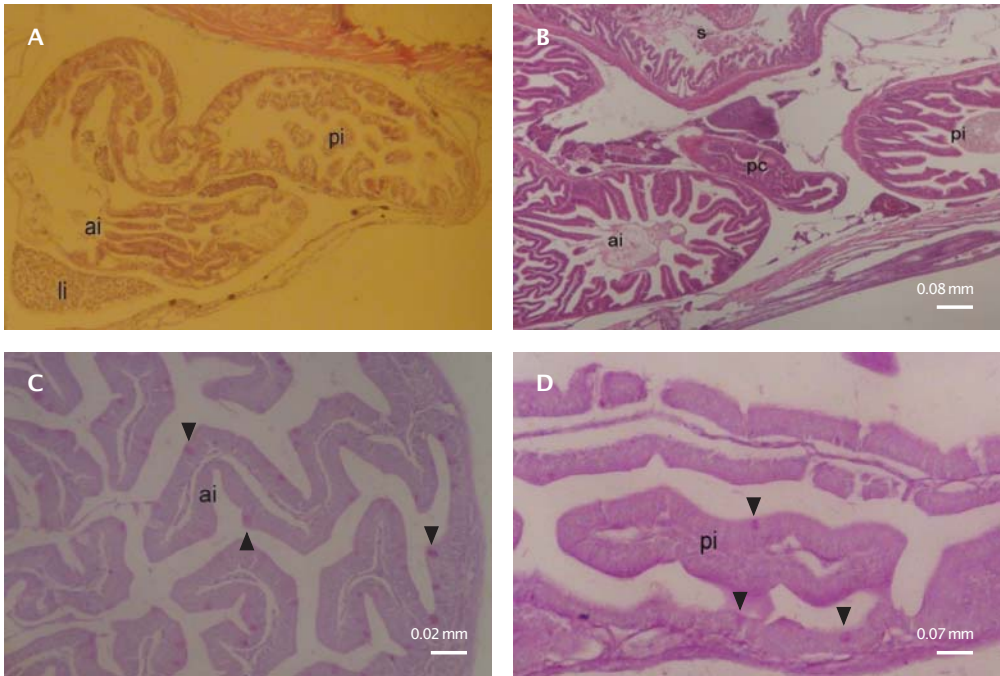


Figure 6. Detail of intestine of larvae at (A) day 12 after hatching showing mucosal folding well developed in the anterior and posterior intestine; HE, 200X. (B) day 30 after hatching showing very prominent mucosal folding; HE, 200X. (C, D) day 30 after hatching with scattered functional goblet cell (arrowheads); PAS, 400X and 600X; liver (li), anterior intestine (ai), posterior intestine (pi), stomach (s), pyloric caecum (pc)

- Liver, pancreas and gall bladder

At hatching, the accessory digestive organs were absent. Liver increased in size after hatching and extended into the space of diminishing yolk sac with the hepatocytes

becoming polyhedral and proliferation of the sinusoids (Fig. 7A). Two types of cytoplasmic inclusions were apparent within hepatocytes by day 12th after hatching: eosinophilic, PAS positive granules and colorless, PAS-negative vacuoles (Fig. 7B). The latter progressively in-

creased in size with larval growth, occupying most of the cytoplasm. At day 2nd after hatching, the incipient pancreas could be detected between liver and intestine, while gall bladder could be detected between liver and the yolk sac wall (Fig. 7A). At day 3rd after hatching, the endocrine cells irregularly shaped with pale cytoplasm and large nucleus, were distinguished as islets of Langerhans inside the exocrine pancreas (Fig. 7D) and well developed by day 20th after hatching (Fig. 7D).

Discussion

Significant changes occurred in the digestive tract of climbing perch larvae during the first several days of development. At hatching, the digestive tract of the larvae was undifferentiated straight tube and rudimentary, similar to the most other fish species reported such as seabass (Walford & Lam, 1993), gilthead seabream (Sarasquete *et al.*, 1995), spotted

sand bass (Pena *et al.*, 2003), California halibut (Gisbert *et al.*, 2004), yellowtail kingfish (Chen *et al.*, 2006), goldlined seabream (Ibrahim *et al.*, 2006), and common pandora (Micale *et al.*, 2006). Differentiation of the digestive tract of climbing perch larvae into four distinct regions, namely, buccopharynx, oesophagus, intestine, and rectum, was established within day 2nd after hatching coinciding with time of the first feeding.

From hatching until to 2 days old, climbing perch larvae depended extremely on an endogenous source of nutrition. According to Heming & Buddington (1988), endogenous nutrition in fish larvae occurred by endocytosis of yolk sac and oil globule through a syncytium surrounding the yolk sac. However, exogenous feeding in climbing perch larvae started before complete re-absorption of yolk sac, as demonstrated by the presence of rotifers and microalgae in the digestive tract by

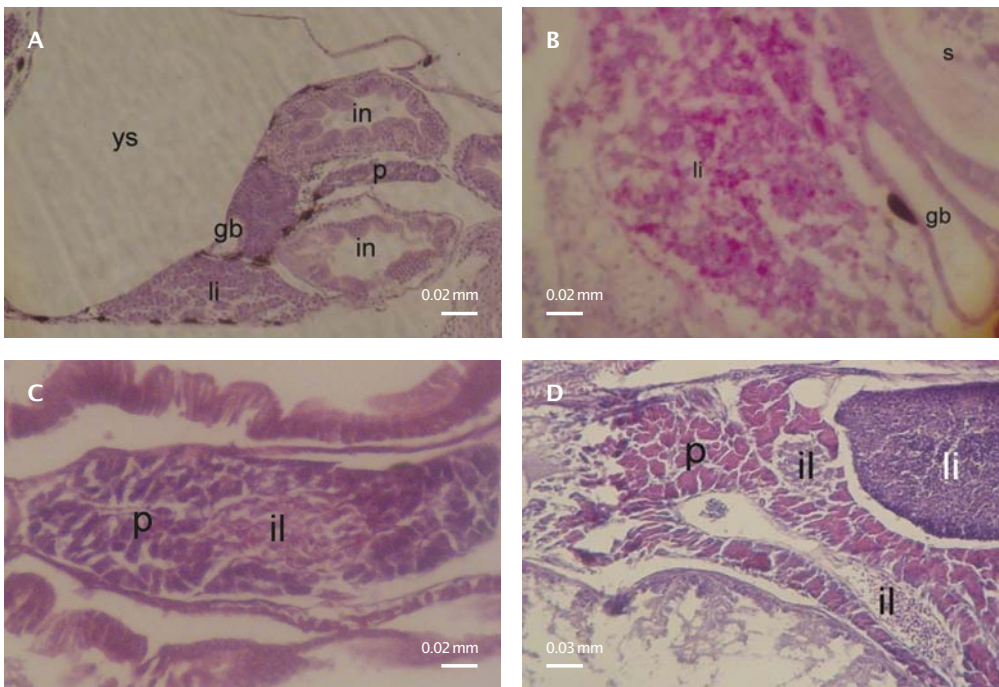


Figure 7. Sagittal sections of climbing perch larvae liver at (A) day 2 after hatching showing gall bladder; HE, 100X. (B) day 12 after hatching showing polygonal hepatocytes containing extensive vacuoles; PAS positive, eosinophilic granules; PAS-negative, colourless vacuoles, 400X. Sagittal section of climbing perch larvae pancreas at (C) day 2 after first feeding showing endocrine cells (islet of Langerhans); HE, 400X, and (D) day 20 after hatching showing well developed; HE, 200X; gall bladder (gb), intestine (in), liver (li), yolk sac (ys), islet Langerhans (il), pancreas (p), swim bladder (sb)

day 2nd after hatching. The use of microalgae and rotifers seemed to be essential during the first stage of climbing perch larvae rearing as well as of the larvae. Indeed, phytoplankton may act as a diet component and/or stimulating factor of digestive enzyme synthesis providing positive effect to the larvae (Reitan *et al.*, 1993).

The liver and pancreas of climbing perch differentiated early in the development process. Two or three days after the first feeding, the presence of pancreas with endocrine cells (islets Langerhans), the larvae may secrete many pancreatic enzymes such as alkaline proteases, amylase, and lipase contributing digestion and absorption of proteins, lipids, and carbohydrates. Moreover, at day 12th after hatching the liver showed a PAS positive reaction with eosinophilic granules as a sign of glycogen deposit indicating functional hepatocytes, whereas the liver showed a PAS negative reaction with colorless vacuoles as a sign of lipid deposit. Boulhic & Gabaudan (1992) suggested that the presence of granules in the enterocytes related to glycogen absorption.

The functional PAS-positive mucous cells in the digestive tract of climbing perch larvae were detected in the pharyngeal and oesophageal epithelium on day 12th after hatching. The late appearance of mucous secretion has been also reported in gilthead seabream (Sarasquete *et al.*, 1995), and California halibut (Gisbert *et al.*, 2004). Glycoproteins produced by goblet cells may play an important lubricant role of the buccopharyngeal and oesophageal mucosa due to lack of salivary glands. Mucosubstances may play important role in protecting digestive mucosa from viral and bacterial attacks (Gisbert *et al.*, 2004).

Intestinal coiling (on day 3rd after hatching) and mucosal folding (on day 12th after hatching) began at a rather early stage and appeared very pronounced in both the anterior and posterior intestines by day 30th after hatching, indicating a better functionality of the gut by the increase of intestinal length and absorption surface. In addition, two pyloric caeca were detected by day 25 after hatching, indicating the presence of functional stomach. Finally, based on the present histological and morphological data, climbing perch larvae could be weaned to formulated feeds on day 25th after hatching.

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