Morphological and Histological Study on the Development of the Digestive System of Siamese Spiny Eel, *Macrognathus siamensis* (Günther, 1861) Larvae

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ABSTRACT

Natural populations of the Siamese spiny eel, *Macrognathus siamensis* (Günther, 1861) have drastically declined while their selling price has been rapidly increasing. For the culture of this species, knowledge of the exact duration of the stages of morphological development of the digestive system would help keepers match appropriate types of food with the changing needs of the larvae. Brood-stocks (10 pairs) of Siamese spiny eel were obtained from the Inland Fisheries Research and Development Regional Center 4 in Ubon Ratchathani Province and artificially induced to spawn by injecting buserelin acetate at 30 $\mu g \cdot kg^{-1}$ and domperidone at 10 $mg \cdot kg^{-1}$. After hatching, 20 fish larvae were serially sampled every two days, from 3 dph (day post hatching) to 40 dph. Fish specimens were fixed in 10% formaldehyde solution and processed for microtome study. Results showed that after hatching, the alimentary canal of the Siamese spiny eel was a straight tube, attached dorsally to the yolk sac. Differentiation of alimentary canal into buccopharynx, esophagus, and anterior and posterior intestine was found at 3 dph, along with the development of other accessory organs, such as liver, pancreas and gall bladder. During 7-9 dph, stomach divided into the cardiac, fundic and pyloric parts. At 11 dph, full development of gastric glands was found along the fundic region, as well as the appearance of the pyloric sphincter, which enabled us to discern the stomach from anterior intestine. From 13 dph until the end of the experiment (40 dph), there was no notable differentiation of the digestive tract except for increasing size. The results suggest that weaning of larvae would be appropriate at 13 dph, according to its fully developed digestive system.

Keywords: Digestive system, Histology, *Macrognathus siamensis*, Morphology, Siamese spiny eel

INTRODUCTION

The Siamese spiny eel or Peacock eel (*Macrognathus siamensis*) is naturally found in canals, streams, swamps, ponds, paddy fields and muddy soil, throughout Thailand, Laos, Cambodia and Vietnam (Rainboth, 1996; Khachonpisitsak, 2007). In Thailand, the species is highly demanded by consumers who are willing to pay 4.96-6.62 $USD \cdot kg^{-1}$ for both live and processed fish. Eels are usually purchased in the market during rainy and rice harvesting seasons. Fish are caught from trap ponds connected to rice fields. The Siamese spiny eel is a medium-sized fish, and the fish are

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a rich source of animal protein, essential fatty acids, vitamins and minerals (Santhanam, 2015). Similar to many other medium-sized fish species in the region, Siamese spiny eels are eaten whole, including head, visceral and bones. They are particularly rich in bioavailable calcium and some are also rich in vitamin A, iron and zinc (Thilsted, 2010). However, the population of this species in natural water bodies has been rapidly decreasing, due to degradation of environments (Khachonpisitsak, 2007). Induced breeding techniques were published in 2007 for this species, by injecting hormone LHRH-a (Luteinizing Hormone Releasing Hormone analog) followed by natural spawning or artificial fertilizations (Saowakoon and Saowakoon, 2007). For the nursing of 1-14 days old Siamese spiny eel larvae, feeding with *Moina macrocopa* obtained the best growth and survival (Saowakoon and Saowakoon, 2007). After 14 dph, the larvae are generally fed with live foods, such as blood worm and earthworms. However, substitution of a prepared diet for live foods is crucial in order to lower production costs and for sustaining a steady production of high quality juveniles (Cahu and Zambonino-Infant, 2001). Understanding the morphology and function of the digestive system in fish larvae is a key requirement in optimizing rearing strategies for a particular species (Zambonino-Infant *et al*., 2008); however, this is lacking for the Siamese spiny eel. Therefore, the purpose of this study was to examine the structural and histological changes of the digestive system in larval Siamese spiny eels until metamorphosis. An understanding of the sequence of organ development will provide baseline information for hatchery management for this species.

MATERIALS AND METHODS

Eggs and larval rearing

Siamese spiny eel broodstock were obtained from the Inland Fisheries Research and Development Regional Center 4 (Ubon Ratchathani). Ten pairs of broodfish kept indoors in a 1,000-L fiberglass tank, were hormonally induced to spawn by intramuscular injections of a synthetic hormone mixtures of 30 $\mu g \cdot kg^{-1}$ buserelin acetate and 10 $mg \cdot kg^{-1}$ domperidone. Induced broodfish were placed in a glass aquarium (46×122×47 cm) with water depth of 40 cm and plastic string plums were provided as substrate for the adhesive eggs. Hatched larvae were stocked at a density of 20 $larvae \cdot L^{-1}$. Water parameters were maintained as follow: temperature 27-29 ºC, dissolved oxygen 4-5 $mg \cdot L^{-1}$, hardness 40-50 $mg \cdot L^{-1}$ $CaCO_3$ and alkalineity 61-70 $mg \cdot L^{-1}$ $CaCO_3$.

Fertilized eggs hatched in approximately 48 h. On the third day of hatching, batches of 1,000 larvae (4.71±0.14 mm, total length; TL) were transferred from brooding aquariums and separately stocked in one of three, 1,000-L tanks. The volume of water in each tank was maintained at 500 L. Yolk sacs were fully absorbed by three days post hatch (dph). After 3 dph larvae were first fed with rotifers (*Brachionus plicatilis*) until 5 dph. Mixtures of rotifers and *Moina macrocopa* were then provided to fish larvae on 5 to 7 dph. After 8 dph only *M. macrocopa* of various sizes were fed until the end of the experiment (40 dph).

Larval handling for morphological and histological analysis

Twenty larvae were randomly sampled every two days (from 3 dph to 40 dph) from rearing tanks for morphological and histological analysis. Total length (TL) was measured, under a microscope to the nearest 0.01 mm on each sampling day and reported as mean total length (TL) from ten fish. Body weights were taken from selected specimens for each experimental batch and fish were released.

On particular sampling days, fish larvae were fixed in 10% phosphate-buffered formalin, thereafter a sub-sample of five larvae were dehydrated in graded ethanol, using automatic tissue processor and embedded in paraffin wax by dispenser. A microtome was used to cut serial sagittal sections (5-6 µm thick), followed by haematoxylin-eosin (H&E) staining following the method of Humason (1979). The tissue sections were then mounted on a slide and observed for general histology following Gentea *et al*. (2009). Tissue examination and imaging were performed by digital camera attached to a light microscope, Nikon model E200.

RESULTS

Morphological development of the digestive tract

After hatching, the digestive tract of the Siamese spiny eel was a straight tube laying dorsally along the yolk sac. At this stage, the mouth and anus of larvae were closed. An oil globule could be seen at the front of the yolk sac. Total length of nearly hatched larval fish averaged 4.71±0.14 mm (0 dph), and increased to 59.25±1.28 mm on 40 dph, whereas average body weights were 3.37±0.06 mg and 496.23±68.59 mg, respectively (Figure 1).

At the age of 2 dph, the yolk sac was partially absorbed, while the mouth was starting to open (344.29±58.27 µm), and the incipient intestine was a straight tube (Figure 2a). According to Saowakoon and Saowakoon (2007), spiny eel start to feed on rotifers at 3 dph, when both mouth and anus are fully opened. The anterior portion of the digestive tube was slightly bent and the intestine-rectal valve was visible (Figure 2b). On 3 dph, the liver was observed in the anterior portion of the digestive tract; also, gallbladder and pancreas appeared. Larva started to feed on rotifers at 4-5 dph, after most of the yolk sac was completely absorbed. At this time, preliminary small conical teeth began to develope on the upper and lower jaws in the mouth.

The loop of the digestive tract was observed in the front part of the abdominal cavity. The incipient intestine was divided into antero-median intestine and rectal area by the intestino-rectal valve (Figure 2c-d). Larvae at 9 dph showed a fully developed digestive tract located in the abdominal cavity (Figure 2e). At 10 dph, the esophagus was narrow and tubular. The anterior portion of the stomach was slightly bent into a V-shape at front rear of the pyloric region. The liver had expanded and fully occupied the anterior portion of the digestive tract (Figure 2f).

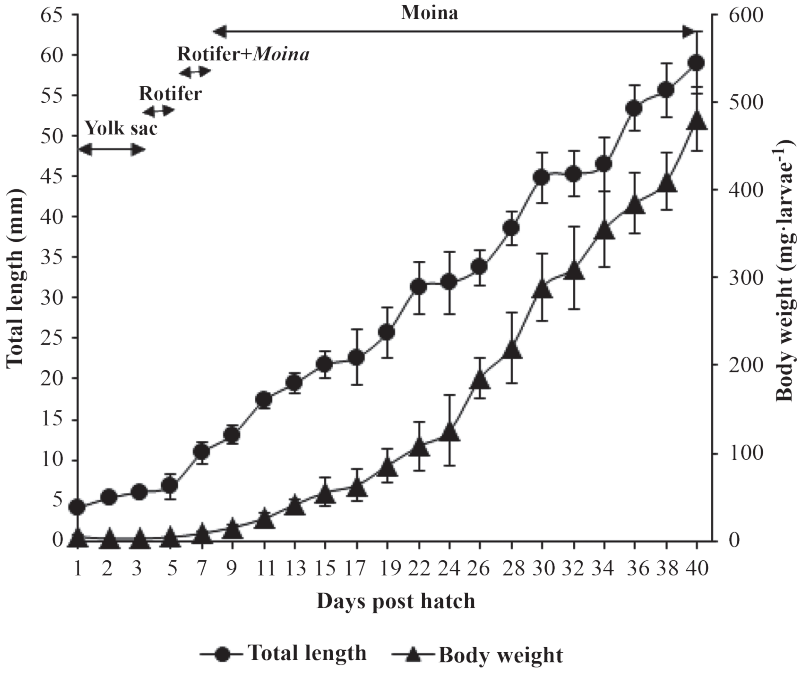

Figure 1. Total length and weight of Siamese spiny eels from 0 to 40 days after hatching. Feeds given are shown above the growth curves.

The morphological transformation of the digestive tube and stomach development, pyloric sphincter and caeca were nearly complete in 13 dph larvae. The tubular cardiac portion of the stomach had spread out behind, and particularly at the bend; it consisted of the long anterior limb, the bend and the short posterior limb, and ended with the pylorus. The pyloric portion was not demarcated externally from the cardiac portion. The pyloric restriction was lifted inside into an annular ridge in the form of truncated cone and would later divide the two lateral pyloric caeca (Figure 2g).

The alimentary canal of the Siamese spiny eel after 16 dph is shown in Figure 2h with a completely transformed digestive tract. In later stages of growth, the stomach grew larger and the pyloric caeca continue to develop. The basic morphological structure of stomach and pyloric caeca did not change after 30 dph.

Buccopharynx

The present histological study showed that in the initial stage, the buccopharyngeal cavity

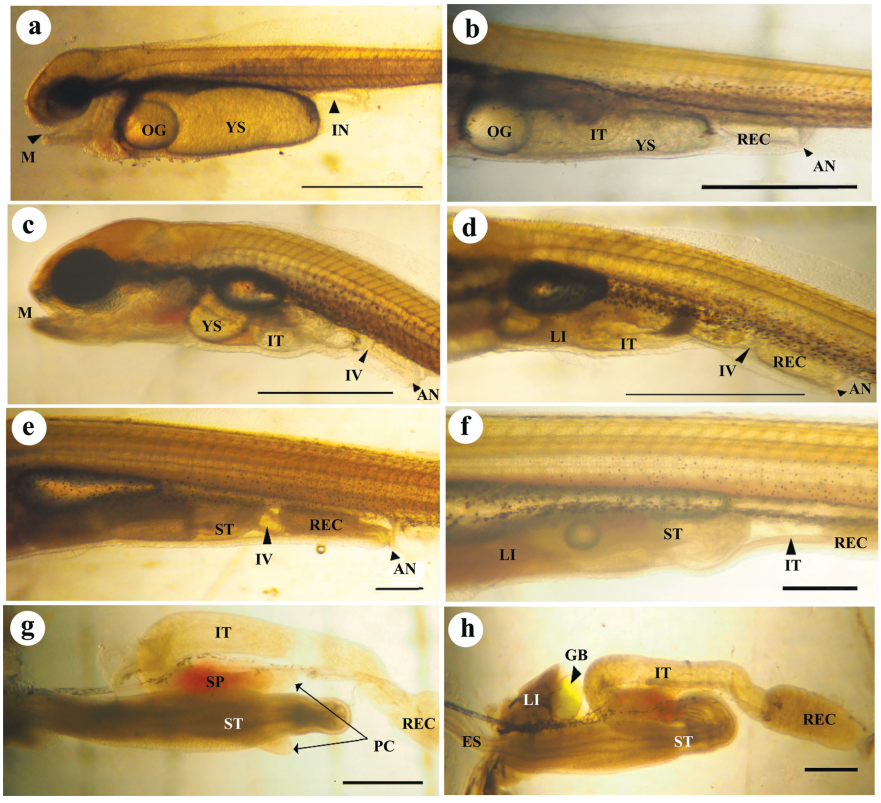

Figure 2. Morphological changes of digestive tract during larval development of Siamese spiny eel: (a) General view of the larvae at 2 dph; (b) Development of the incipient intestine at 3 dph; (c,d) Lateral view of the digestive tract at 4 and 5 dph; (e,f) View of the digestive tract at 9 and 10 dph; (g) Pyloric caeca (arrows) between the pyloric stomach and anterior intestine at 13 dph; (h) General structure of the digestive system at 16 dph. AN = anus; ES = esophagus; GB = gall bladder; IN = incipient intestine; IT = intestine; IV = intestinal valve; LI = liver; M = mouth; OG = oil globue; PC = pyloric caeca; REC = rectum; ST = stomach; SP = spleen; YS = yolk sac. Scale bar = 1 mm.

was lined by a single layer of simple cuboidal epithelium, which later formed a stratified cuboidal epithelium at 4 dph (Figure 3a-b). On 5 dph, teeth emerged on the upper and lower pharyngeal jaws. Taste buds, oral valves as well as mucous cells formed among the epithelium cells (Figure 3c-d). The number of taste buds, pharyngeal teeth and mucous cells dramatically increased at 7 dph (Figure 3e). No apparent histological change was observed in the buccopharynx after 7 dph onward to the end of study at 40 dph.

Esophagus

At 3 dph, the esophagus was a narrow duct with uneven lumen. It was located between the posterior part of the pharynx and anterior intestine. The epithelial cells were composed of mucous cells and lined with simple cuboidal epithelium (Figure 4a). During 7 and 9 dph, the muscular layer of esophagus was surrounded by inner longitudinal and outer circular muscle and mucosal folds which could be observed at the posterior part of the esophagus

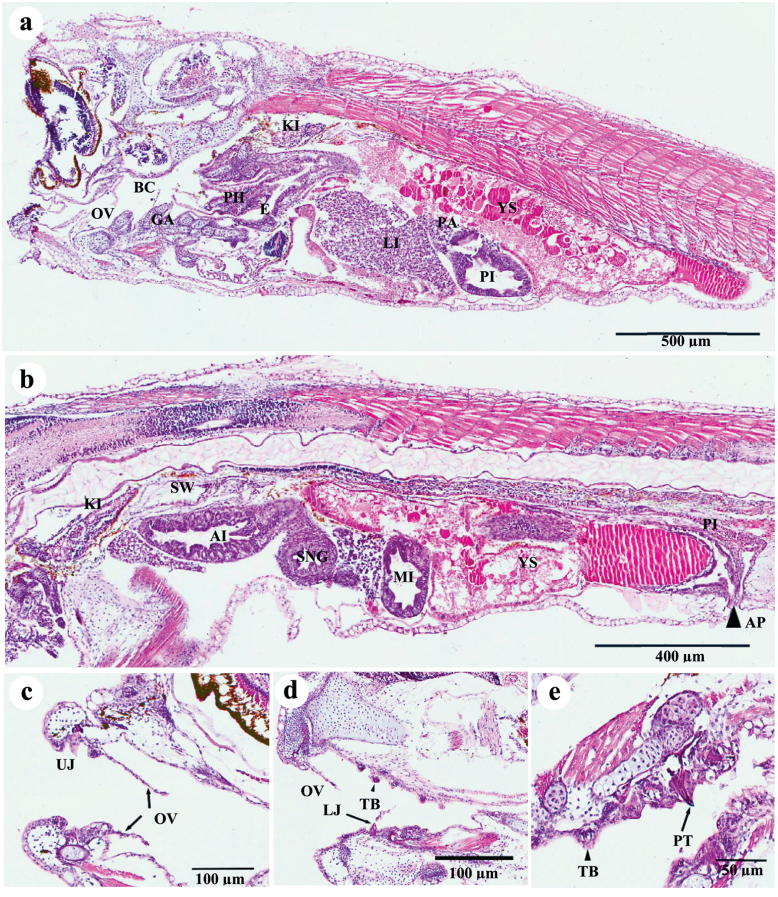

Figure 3. Histological sections of the buccopharynx of Siamese spiny eel larvae at different developmental stages: (a,b) Sagittal sections at 3 dph; (c,d) Sagittal sections of the buccopharynx cavity at 5 dph; (f) Buccopharynx cavity showing the presence of pharyngeal teeth at 7 dph; AI = anterior intestine; AP = anus pore; BC = buccopharyngeal cavity; E = esophagus; GA = gill arches; KI = kidney; LI = liver; LJ = lower jaw teeth; MI = median intestine; NGS = non-glandular stomach; OV = oral valve; PA = pancreas; PH = pharynx; PI = posterior intestine; PT = pharyngeal teeth; SW = swim bladder; TB = taste bud; UJ = Upper jaw teeth; YS = yolk sac.

(Figure 4b). From 11 to 13 dph, this muscle layer became thicker. Goblet cells and fat cells were rapidly increasing in number. After 30 dph, the esophagus did not show histological change until the end of the study.

Stomach

The stomach emerged as a bulge at the posterior part of the esophagus at 3 dph. Columnar cells appeared on the wall of the digestive tube. The anterior part of the non-glandular stomach developed into the pyloric portion of the stomach, and its epithelium was lined with columnar cells containing basal nuclei (Figure 5a). During 7 to 9 dph, the stomach enlarged in size. The mucosal folds increased in number, while the brush border was observed in the mucosal epithelium (Figure 5b). The non-glandular stomach became separated from the anterior part of intestine by the mucosal fold. At this stage, the stomach then differentiated into three parts: cardiac, fundic and pyloric regions (Figure 5e). The cardiac region was seen at the anterior part of the stomach, and occupied by several mucosal folds lined by simple cuboidal epithelium but lacking mucous cells. The fundic region was the largest portion of the stomach and consisted of gastric tubular which changed into groups of rectangular cells. The last portion of stomach was the pyloric region. This section was rather short and rich in connective tissue and led to increased muscular fibres in the pyloric sphincter. During 9 to 11 dph, the non-glandular stomach was differentiated into glandular stomach containing gastric tubular glands (Figure 5c) consisting of secretory cells, increased by numerous longitudinal folds. The microvilli at the apical border were lined with a simple cuboidal epithelium (Figure 5d).

The glandular layer of the stomach consisted of the lamina propria, submucosa and muscular layer. The muscular layer contained two smooth muscle layers, inner circular and outer longitudinal muscle. The transverse inner layer (circular muscle) was thicker than the outer layer (longitudinal muscle). From 13 to 20 dph, the size of the stomach increased with age, and mucosal folds in both fundic and pyloric regions were multiplied in number. The number of gastric glands increased with age along with their increased prominence beneath the epithelium of the fundic region, but gastric glands did not appear in the pyloric region. At 35-40 dph, the stomach was completely developed and formed the largest portion of the abdominal cavity.

Intestine

The incipient intestine of larvae was long, narrow and was the first organ of the digestive system to be differentiated (Figure 6a). During 3 and 4 dph, the intestinal sphincter developed between midgut and hindgut of the intestinal tract. At 5 dph, the intestinal mucosal folds were elevated and enlarged on the mucosa. From 7 to 9 dph, mucosal folds of the anterior part of intestinal valve became thicker compared to larvae at 5 dph

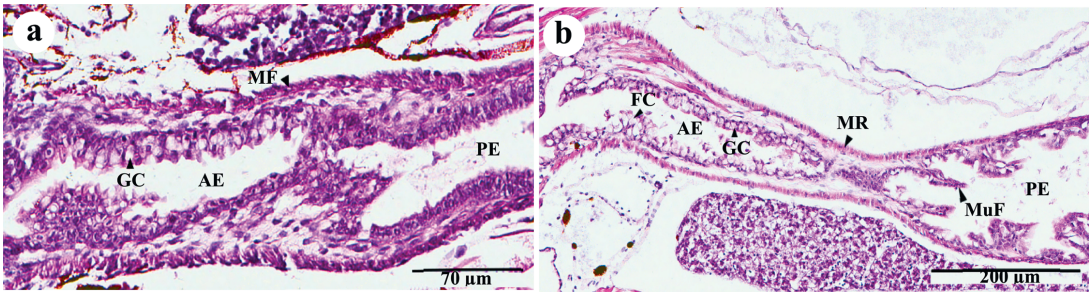

Figure 4. Histological section of the esophagus at different development stages of Siamese spiny eel larvae: (a) Sagittal section at 3 dph; (b) 7 dph; AE = anterior esophagus; FC = fat cells; GC = goblet cell; LI = liver; MF = muscular fibers; MR = muscularis; MuF = mucosal folds; PE = posterior esophagus.

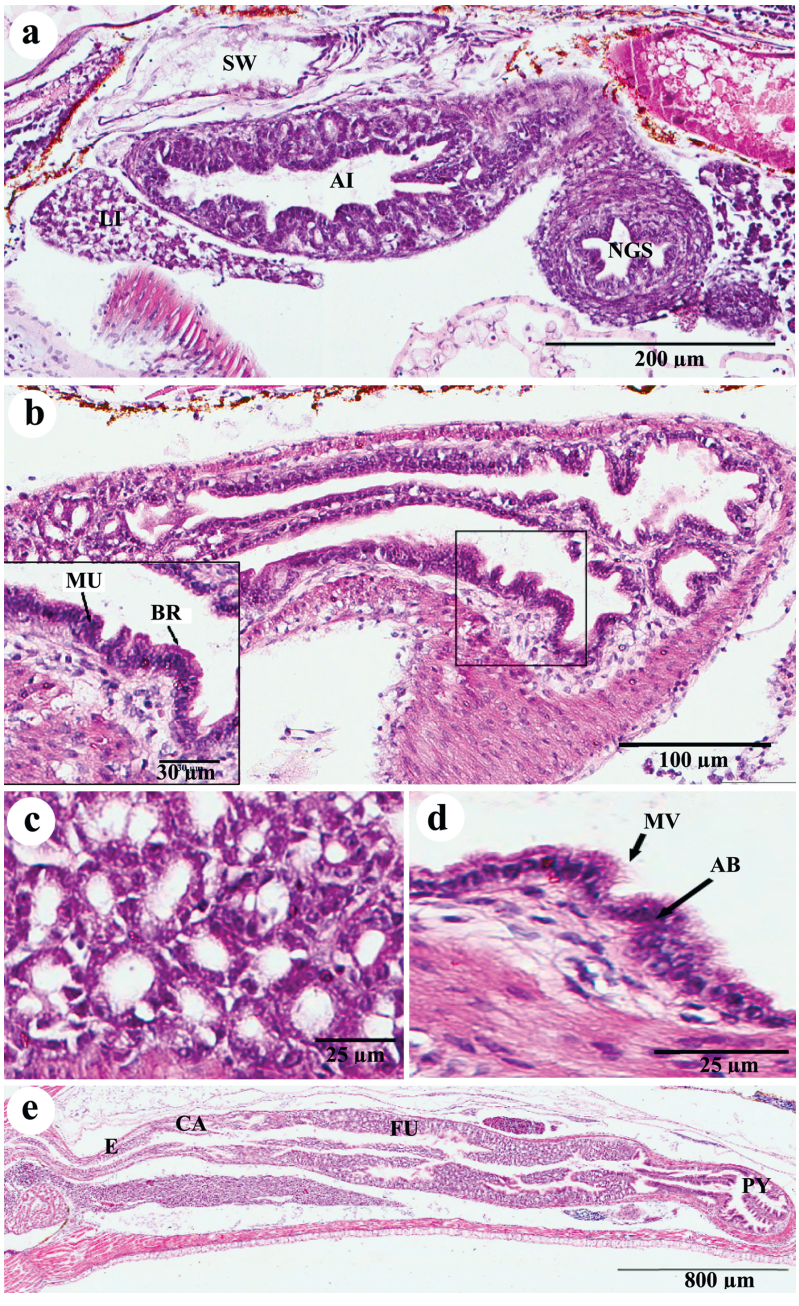

Figure 5. Histological sections of stomach of Siamese spiny eel larvae at different developmental stages: (a) General view of the stomach at 3 dph; (b) Histological section of stomach at 7 dph showing mucosal folds and brush border; (c) Gastric glands at 11 dph; (d) Detail of the microvilli and apical border at 11 dph; (e) Sagittal section of cardiac, fundic and pyloric regions at 13 dph; AB = apical border; AI = anterior intestine; BR = brush border; CA = cardiac; FU = fundic; LI = liver; MU = mucosa folds; MV = Microvilli; NGS = non-glandular stomach; PA = pancreases; PY = pyloric; SW = swim bladder; YS = yolk sac.

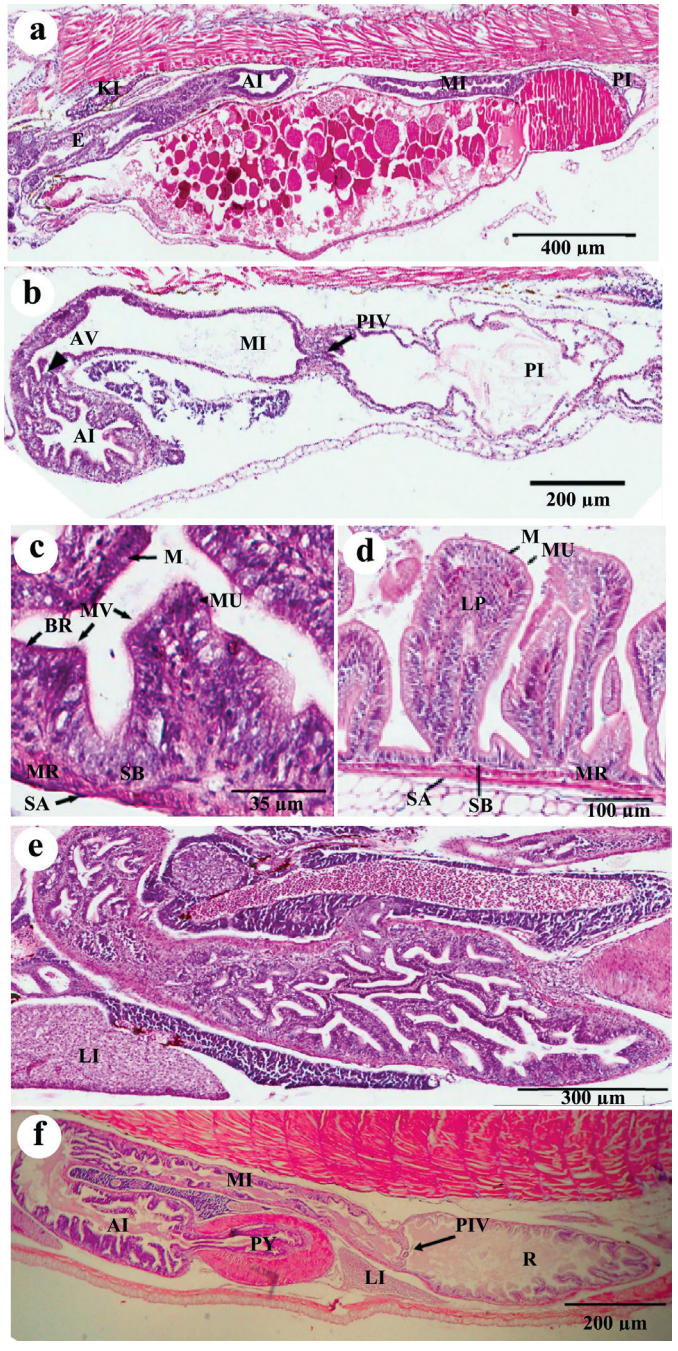

Figure 6. Histological sections of intestine at different stages of Siamese spiny eel larvae : (a) 3 dph; (b) intestinal valve, anterior, middle and posterior mucosae at 7 dph; (c) epithelium of the antero-median intestine at 17 dph; (d) epithelium of rectum intestine at 35 dph; (e) pyloric caeca at 17 dph; (f) histological section of intestine at 17 dph; AI = anterior intestine; AV = anterior intestinal valve; BR = brush border; E = esophagus; KI = kidney; LI = liver; LP = lamina propia; M = mucous glands; MI = median intestine; MR = mucularis; MU = mucosa layer; MV = microvilli; PA = pancrease; PI = posterior intestine; PIV = posterior intestinal valve; PY = pyloric sphincter; R = rectum; SA = serosa; SB = submucosa; YS = yolk sac.

and also enlarged in size (Figure 6b). Between 11 and 22 dph, intestinal mucous glands emerged between the intestinal columnar epithelium and the brush border. Meanwhile, microvilli appeared on the mucosal folds (Figure 6c), and the pyloric region started to form the pyloric sphincter at 11 dph.

At 17 dph, a pyloric caeca with two lateral appendages was observed, located at the anterior part of the intestine and pyloric sphincter regions (Figure 6e). The rectum appeared short, connected to the middle intestinal segment and devoid of mucosal folds (Figure 6d, f). The thin muscular layer of the intestinal mucosa was composed of internal circular and external longitudinal muscular tissue. After 22 dph, no major changes were observed, except for the increase of the digestive tract size (Figure 6f).

Accessory organs

Liver

During the hatching stage, the liver was only a cluster of round cells behind the yolk sac. At 3 dph, the liver was observed as two separate sections, covering the front part of the digestive tract below the yolk sac (Figure 7a). The liver cells consisted of basophilic bodies with nuclei located at the margin of cells, owing to presence of lipid vacuoles which pushed the nucleus to edge of the cell (Figure 7a-b). By 9 dph, two lobes of the liver began to cover the esophagus and gallbladder. The shape of hepatocytes became polyhedral, with round nucleus and granular cytoplasm. Red blood cells were found in the sinusoid (Figure 7c). As larval age increased, the liver and sinusoid became larger. After 35 dph, the liver was completely developed, and its size continued to increase with age.

Pancreas

At 3 dph, the pancreatic cells were elongated and they were spread between the liver and the intermediate intestine (Figure 7a). Pancreas cells were composed of exocrine tissue, distributed around the membrane of the gastrointestinal tract. Acidophilic zymogen granules were round-shaped, with eosinophils grouped in the center of the acini (Figure 7d-e). During 9-11 dph, the endocrine cells (islets of Langerhans) were surrounded by exocrine cells (Figure 7f). The exocrine pancreatic cells consisted of groups of 5-8 polyhedral cells called lobular acini. In addition, the duct of the interlobular pancreas was lined with cuboidal epithelium in the exocrine tissue and opened to the ventral part of the anterior intestine. After 35 dph, the pancreas was morphologically developed, while the organ increased in size along with fish size.

Gallbladder

The gallbladder was observed as an oval shaped at 3 dph and became fully developed by 7 dph. During this period, this organ consisted of simple cubic cells surrounded by a layer of connective tissue (Figure 7g). At 13 dph, the epithelium of the gallbladder became thickened with cuboidal and cylindrical cells surrounded by hepatocytes and pancreatic cells (Figure 7h). A basal lamina of connective tissue was defined smooth muscle cells underlying the epithelium.

DISCUSSION

An understanding of the development of the digestive tract of fish larvae is important for management of larval growth and survival. For aquaculture, it is necessary for preparing the types of food in accordance with organ development, and for adjusting the type of food at the appropriate time (Cañavate and Fernández-Dıaz, 1999; Cyrino *et al*., 2008). Morphologically, development of the digestive tract of Siamese spiny eel larvae was similar to other species, such as the Senegal sole *Solea senegalensis* (Sarasquete *et al*., 1996), the common pandora fish *Pagellus erythrinus* (Micale *et al*., 2006), and the European eel *Anguilla anguilla* (Knutsen, 2015). At hatching stage, the gastrointestinal tract is straight, while mouth and anus are not yet opened. However, there are many factors associated with the development of digestive tracts of fish larvae, including genetics, quality of eggs, food quality, temperature etc. (Zambonino-Infante *et al*., 2008).

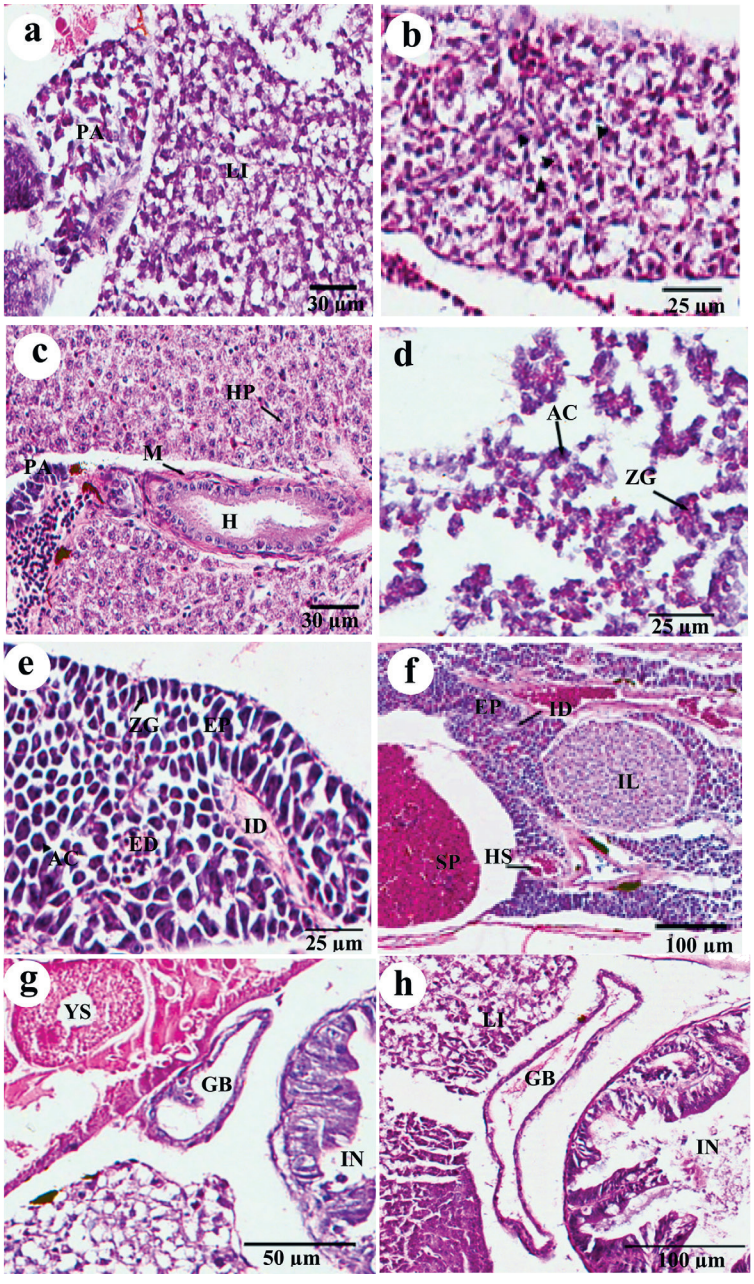

Figure 7. Histological sections of liver, pancreatic and gallbladder tissue of Siamese spiny eel larvae at different stages development. (a) sagittal section at 3 dph showing hepatic cells in liver; (b) lipid vacuoles (▲) within hepatocytes at 11 dph; (c) liver tissue completely developed at 35 dph; (d) pancreatic tissue section at 5 dph; (e) polyhedral basophilic parenchyma arranged in acini containing zymogen granules at 11 dph; (f) pancreatic tissue section at 35 dph, showing exocrine pancreas and islets of Langerhans; (g) gallbladder at 3 dph; (h) gallbladder at 13 dph; AC = acinar cells; ED = endocrine pancreas; EP = exocrine pancreas; H = hepatic bile duct; HP = hepatocytes; HS = hepatic sinusoid; ID = interlobular duct; IL = islet of Langerhan (endocrine pancreas); LI = liver; M = smooth muscle; PA = pancreas; SP = spleen; ZG = zymogen granules; GB = gallbladder; IN = intestine; YS = yolk sac.

In this study, it was found that Siamese spiny eel larvae from hatching until early 3 dph relied exclusively on their yolk reserves, while a period of mixed nutrition based on endogenous reserves and live prey (*Moina*), was observed during 3-4 dph. In tropical fish, the opening of the mouth and final use of the yolk sac occur within a short period of time. Most fish larvae open their mouths before the yolk sac is fully absorbed (Thalathiah *et al*., 1988; Smith, 1989; Jaroszewska and Dabrowski, 2011; Pradhan *et al*., 2012; Rangsin *et al*., 2012). The major developmental events of the digestive system in Siamese spiny eel are summarized in a timeline presented in Figure 8. Opening of mouth and anus, indicate readiness for accepting exogenous food; therefore, providing rotifers to spiny eel larvae should begin at 3 dph (Figure 1).

Development of the buccopharynx, was observed as the first stage after hatching. The mouth of the Siamese spiny eel larvae was still closed, while both upper and lower jaw were still forming in the dermis layer. At this stage, the buccopharynx was surrounded by thin connective tissue and the hypodermis layer in the oral cavity, and would be completed by 3 dph. Similar results were reported in the featherback *Chitala chitala* (Mitra *et al*., 2015), striped murrel *Channa striata* (Paray *et al*., 2015) and Amazonian pimelodid catfish, *Pseudoplatystoma punctifer* (Gisbert *et al*., 2014); their mouths are fully opened during 2 to 4 dph.

The teeth on the upper and lower jaws emerged at 5-7 dph, earlier than the presence of pharyngeal teeth, which appeared during larval metamorphosis. The pharyngeal cavity was lined with simple cuboidal epithelium and surrounded by connective tissue and circular muscle. Development of a tongue occurred at the mouth floor, recognized by stratified cuboidal epithelium, similar to the redbanded seabream, *Pagrus auriga* (Sánchez-Amaya *et al*., 2007), flatfish brill fish *Scophthalmus rhombus* (Hachero-Cruzado *et al*., 2009) and tropical gar fish, *Atractosteus tropicus* (Frías-Quintana *et al*., 2015). In the present study, goblet cells in the esophagus were first observed at 3 dph, along with increase of stratified epithelium. Furthermore, goblet cells and taste buds were found to be abundant in the buccopharyngeal mucosa. According to Kapoor *et al*. (1975), these goblet cells help fish to facilitate the passage of food through the esophagus, which might be a good indicator of the ability to feed on exogenous food.

The esophagus in spiny eel larvae was a short tube with narrow duct lined by cuboidal epithelium connecting the buccopharyngeal cavity to the anterior of the intestine. Siamese spiny eel larvae goblet cells were first observed at 3 dph, the same time as in several other species, such as the Senegal sole, *Solea senegalensis* (Ribeiro *et al*., 1999), redbanded seabream *Pagrus auriga* (Sánchez-Amaya *et al*., 2007), butter catfish larvae, *Ompok bimaculatus* (Pradhan *et al*., 2012) and

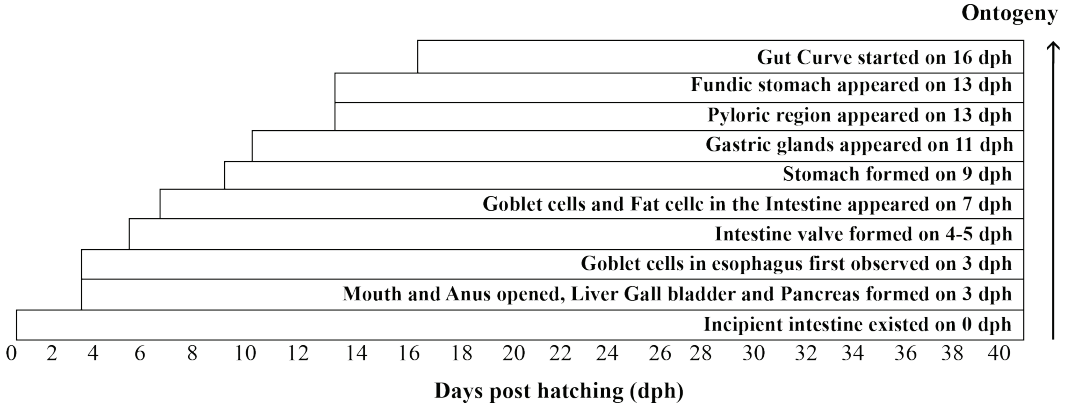

Figure 8. Summary of major developmental events in the digestive system ontogeny of Siamese spiny eel lavae, from hatching to 40 days post hatching (dph).

Pacific red snapper (Peña *et al*., 2017). This coincided with opening of the mouth and ability to feed on exogenous food. On the other hand, in other species such as California halibut, *Paralichthys californicus* (Gisbert *et al*., 2004), goblet cells appeared between 9 and 11 dph, at the onset of exogenous feeding. Abundance of goblet cells in the posterior region of the buccopharyngeal and esophagus, would indicate that the fish could secrete lubricant to protect the buccopharyngeal and esophageal mucosa from abrasion while ingesting preys (Gisbert *et al*., 1999).

The stomach of the Siamese spiny eel could be morphologically distinguished soon after hatching during the conversion stage of the esophagus and intestine. Thereafter, the stomach was divided into three regions: cardiac, fundic and pyloric regions. The stomach was then composed of a cluster of cuboidal to columnar epithelium cells with a sub-epithelial connective layer, and surrounded by a circular muscle layer with a thin tunica serosa. At 7 dph, the gastric glands as tubular glands arranged along numerous mucosal folds within the fundic region. Presence of gastric glands was observed between 8 and 10 dph, a few days after first feeding on live food, similar to the Siberian sturgeon, *Acipenser baeri* (Gisbert *et al*., 1999) shi drum, *Umbrina cirrosa* (Zaiss *et al*., 2006) cobia, *Rachycentron canadum* (Faulk *et al*., 2007) and butter catfish, *Ompok bimaculatus* (Pradhan *et al*., 2012). However, gastric glands of spiny eel developed faster than other fish species such as Senegalese sole, *Solea senegalensis* (Ribeiro *et al*., 1999), common Pandora, *Pagellus erythrinus* (Micale *et al*., 2006), yellowtail kingfish, *Seriola lalandi* (Chen *et al*., 2006), flatfish brill, *Scophthalmus rhombus* (Hachero-Cruzado *et al*., 2009), and Amazonian pimelodid catfish, *Pseudoplatystoma punctifer* (Gisbert *et al*., 2014).

According to Rønnestad *et al*. (2013), the appearance of gastric glands is a sign of the ability to digest live food, as well as the onset of digestive enzymes action. The stomach is probably functional in larvae of fish, since neutral muco-substances can protect the stomach from auto-digestion by HCl and enzymes produced by gastric glands. This is a crucial event in enabling young fish to digest food, and may represent the point at which we can wean spiny eel larvae from live food to artificial diets, reducing production costs. The differentiation of the pyloric caeca in spiny eel appeared during 9 to 10 dph, coinciding with development of gastric glands in the stomach, which is similar to the turbot, *Scophthalmus maximus* (Cousin and Laurencin, 1985). This might be a good indicator of the completion of the larval period and the transition to the juvenile stage. As reported by Stroband and Kroon (1981), development of the gastrointestinal tract and pyloric caeca is highly correlated with the change of gastric glands in order to enable gastric function.

The intestine of the Siamese spiny eel larvae, originating from the pyloric sphincter, was the longest portion of the digestive tract. Histologically, the appearance of the intestine of newly hatched fish was similar to observations in featherback, *Chitala chitala* (Mitra *et al*., 2015), as their intestines were divided into midgut and hindgut by an intestinal valve. After 5 dph, the intestine had divided into three region: anterior, middle and posterior region at the histological level. Though there was no morphological differences observed which same as the fishes. At 10 dph the folded mucosal epithelium of the anterior and posterior intestine consisted of absorptive columnar cells with a distinct brush-like border of microvilli scattered among the goblet cells. Similar results have been reported in Asian seabass, *Lates calcarifer* (Walford and Lam, 1993) and European whitefish, *Coregonus lavaretus* (Ostaszewska *et al*., 2018). Machado *et al*. (2013) proposed that an increased number of goblet cells in the rectum, a common feature in fishes, is probably useful for increased mucous production in order to secure the intestinal lining and acid in fecal expulsion. This phenomena was also reported in the African catfish, *Clarias gariepinus* (Verreth *et al*., 1992) and rice field eel, *Monopterus albus* (Dai *et al*., 2007).

After hatching, the liver and pancreas appeared as a group of moderate sized, undifferentiated cells. Between 2 and 3 dph, the incipient liver appeared as two groups of basophilic

cells lying between the digestive tract and the yolk sac. Similar findings were reported in several teleost species, such as sea bream (Guyot *et al*., 1995) and Senegal sole (Sarasquete *et al*., 1996). After 3 dph, the liver was divided into two lobes and began to differentiate by substantially increasing the number of hepatocytes. Similar results were recorded in the striped murrel, *Channa striatus* (Paray *et al*., 2015), and butter catfish, *Ompok bimaculatus* (Pradhan *et al*., 2012). From our study it was observed that at 5 dph, the size of the liver had fully extended as a result of hepatocyte differentiation and increasing number of lipid vacuoles. Formation of internal bile ducts of spiny eel larvae is probably concurrent with the gradual maturation of hepatocytes, which is in accordance with Hoehne-Reitan *et al*. (2001) who reported that liver functions were to synthesize, store and mobilize carbohydrates and lipids.

At 3 dph, the pancreas of the Siamese spiny eel started to develop pancreatic cells located above the liver and diffused over the intermediate intestine. The pancreatic cells composed of zymogen granules at the center of the acinar cells were similar to observations in the gilt-head sea bream, *Sparus aurata* (Guyot *et al*., 1998). Moreover, the exocrine pancreas cells were concentrated in acini, as pancreatic ducts also appeared on 3 dph. However, this was quite different from the red banded seabream, *Pagrus auriga*, in which the zymogen granules were first observed at 21 dph, before onset of first feeding (Sánchez-Amaya *et al*., 2007). This difference might be an affect of difference in ambient temperature. Between 9 and 11 dph, the exocrine pancreas was spread around the endocrine pancreas (islet of Langerhans). The gallbladder was first observed on 3 dph as an oval shaped sac, located between liver and yolk sac. The gallbladder can be recognized as a simple cuboidal layer surrounded by connective tissue. Similar to some other fish species, gallbladders were first detected at 3 dph, such as in common Pandora, *Pagellus erythrinus* (Micale *et al*., 2006) redbanded seabream, *Pagrus auriga* (Sánchez-Amaya *et al*., 2007), and striped murrel, Channa striata (Paray *et al*., 2015).

In conclusion, our morphology and histology studies of the digestive tract of the Siamese spiny eel (*Macrognathus siamensis*) larvae revealed that the basic development pattern of the digestive tract is similar to other freshwater fish. The digestive organ formation was complete when gastric glands and pyloric caeca appeared, between 9 and 13 dph. Results from our study suggest that a suitable time to change from live food to an artificial diet is at 13 dph, due to the full development of the fundic part of the stomach. Further research on the digestive system of this species should emphasized histochemical and biochemical aspects, including digestive enzymes, as well as weaning strategies for better understanding of the functionality of the digestive tract during ontogenetic development.

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LITERATURE CITED

Cahu, C.L. and J.L. Zambonino-Infante. 2001. Substitution of live food by formulated diets in marine fish larvae. **Aquaculture** 200(1–2): 161–180.

Cañavate, J.P. and C. Fernández-Dıaz. 1999. Influence of co-feeding larvae with live and inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds. **Aquaculture** 174(3–4): 255–263.

Chen, B.N., J.G. Qin, M.S. Kumar, W.G. Hutchinson and S.M. Clarke. 2006. Ontogenetic development of digestive enzymes in yellowtail kingfish *Seriola lalandi* larvae. **Aquaculture** 260(1–4): 264–271.

Cousin, J.C.B. and F.B. Laurencin. 1985. Morphogenesis of the digestive system and swim bladder of the turbot, *Scophthalmus maximus* L. **Aquaculture** 47(4): 305–319.

Cyrino, J.E.P., D.P. Bureau and B.G. Kapool. 2008. **Feeding and digestive functions in fishes.** CRC Press, Florida. 597 pp.

Dai, X., M. Shu and W. Fang. 2007. Histological and ultrastructure study of the digestive tract of rice field eel, *Monopterus albus*. **Journal of Applied Ichthyology** 23(2): 177–183.

Faulk, C.K., A.D. Benninghoff and G.J. Holt. 2007. Ontogeny of the gastrointestinal tract and selected digestive enzymes in cobia *Rachycentron canadum* (L.). **Journal of Fish Biology** 70(2): 567–583.

Frías-Quintana, C.A., G. Márquez-Couturier, C.A. Alvarez-González, D. Tovar-Ramírez, H. Nolasco-Soria, M.A. Galaviz-Espinosa and E. Gisbert. 2015. Development of digestive tract and enzyme activities during the early ontogeny of the tropical gar *Atractosteus tropicus*. **Fish Physiology and Biochemistry** 41(5): 1075–1091.

Gentea, F., E. Terwinghe and A. Danguy. 2009. **Atlas of fish histology.** Science Publishers, New Hampshire, USA. 215 pp.

Guyot, E., J.P. Diaz and R. Connes. 1995. Organogenesis of the liver in sea bream. **Journal of Fish Biology** 47(3): 427–437.

Guyot, E., J.P. Diaz, B. Romestand and R. Connes. 1998. Insulin during the early postembryonic development of gilt-head sea bream, *Sparus aurata*: ultrastructural, immunohistochemical, and biochemical studies. **General and Comparative Endocrinology** 110: 146–156.

Gilbert, E., R.H. Piedrahita and D.E. Conklin. 2004. Ontogenetic development of the digestive system in California halibut (*Paralichthys californicus*) with notes on feeding practices. **Aquaculture** 232(1): 455–470.

Gilbert, E., M.C. Sarasquete, P. Williot and F. Castelló-Orvay. 1999. Histochemistry of the development of the digestive system of Siberian sturgeon during early ontogeny. **Journal of Fish Biology** 55(3): 596–616.

Gisbert, E., C. Moreira, D. Castro-Ruiz, S. Öztürk, C.F. Mendez, S. Gilles, J. Núñez-Rodriguez, F. Duponchelle, S. Tello, J.F. Renno, C. Davila and M. Darias. 2014. Histological development of the digestive system of the Amazonian pimelodid catfish *Pseudoplatystoma punctifer*. **Animal** 8(11): 1765–1776.

Hachero-Cruzado, I., J.B. Ortiz-Delgado, B. Borrega, M. Herrera, J.I. Navas and C. Sarasquete. 2009. Larval organogenesis of flatfish brill *Scophthalmus rhombus* L: Histological and histochemical aspects. **Aquaculture** 286(1): 138–149.

Hoehne-Reitan, K., E. Kjørsvik and D.R. Gjellesvik. 2001. Development of bile salt-dependent lipase in larval turbot. **Journal of Fish Biology** 58(3): 737–745.

Humason, G.L. 1979. **Animal tissue technique.** W.H. Freeman and Company. San-Francisco. 661 pp.

Jaroszewska, M. and K. Dabrowski. 2011. **Utilization of yolk: transition from endogenous to exogenous nutrition in fish.** In: Larval fish nutrition (ed. G.J. Holt), pp. 183–218. Wiley-Blackwell, Oxford.

Kapoor, B.G., H. Smith and I.A. Verighina. 1975. The alimentary canal and digestion in teleosts. **Advances in Marine Biology** 13: 109–239.

Khachonpisitsak, S. 2007. **Taxonomy of spiny eels (Symbranchiformes: Mastacembelidae) in Thailand.** Master's Thesis, Chulalongkorn University, Bangkok, Thailand. 260 pp.

Knutsen, H.R. 2015. **Morphological development of wild leptocephalus larvae of the European eel (*Anguilla anguilla*)-with special emphasis on muscle and digestive system.** https://brage.bibsys.no/xmlui/handle/11250/2358867. Cited 3 Jan 2018.

Machado, M., H. Souza, V. Lemos de Souza, A. De Azevedo, R. Goitein and A.D. Nobre. 2013. Morphological and anatomical characterization of the digestive tract of *Centropomus parallelus* and *C. undecimalis*. **Acta Scientiarum-Biological Sciences** 35: 467–474.

Micale, V., M. Garaffo, L. Genovese, M.T. Spedicato and U. Muglia. 2006. The ontogeny of the alimentary tract during larval development in common pandora *Pagellus erythrinus* L. **Aquaculture** 251(2–4): 354–365.

Mitra, A., K.M. Pratap and H. Sumit. 2015. Histomorphological study of the gut developmental pattern in early life histology stages of featherback, *Chitala chitala* (Hamilton). **Archives of Polish Fisheries** 23: 25–35.

Ostaszewska, T., K. Krajnik, D. Adamek-Urbańska, R. Kasprzak, M. Rzepkowska, M. Luczynski and K. Dabrowski. 2018. Effect of feeding strategy on digestive tract morphology and physiology of lake whitefish (*Coregonus lavaretus*). **Aquaculture** 497: 32–41.

Paray, B.A., M.A. Haniffa, M.U.D. War, M.K. Al-Sadoon, Y.H. Park and I.A. Rather. 2015. Histological changes in the digestive tract of striped murrel larvae during ontogeny. **Indian Journal of Geo-Marine Sciences** 44(7): 984–992.

Peña, R., S. Dumas and M. Contreras-Olguín. 2017. Organogenesis of the digestive system in Pacific red snapper (*Lutjanus peru*) larvae. **Aquaculture Research** 48(4): 1561–1575.

Pradhan, P., K.J. Jena, G. Mitra, N. Sood and E. Gisbert. 2012. Ontogeny of the digestive tract in butter catfish *Ompok bimaculatus* (Bloch) larvae. **Fish Physiology and Biochemistry** 38: 1601–1617.

Rainboth, W.J. 1996. **Fishes of the Cambodia Mekong.** Food and Agriculture Organization of The United States. Rome. 265 pp.

Rangsin, W., N. Areechon and R. Yoonpundh. 2012. Digestive enzymes activities during larval development of striped catfish, *Pangasianodon hypopthalmus* (Sauvage, 1878). **Kasetsart Journal: Natural Science** 46: 217–228.

Rønnestad, I., Y. Manuel, U. Bernd, R. Laura, S. øystein and B. Clara. 2013. Feeding behavior and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. **Reviews in Aquaculture 5** (Suppl. 1): 59–98.

Ribeiro, L., C. Sarasquete and M.T. Dinis. 1999. Histological and histochemical development of the digestive system of *Solea senegalensis* (Kaup, 1858) larvae. **Aquaculture** 171(3–4): 293–308.

Sánchez-Amaya, M.I., J.B. Ortiz-Delgado, Á. García-López, S. Cárdenas and C. Sarasquete. 2007. Larval ontogeny of redbanded seabream *Pagrus auriga* Valenciennes, 1843 with special reference to the digestive system. A histological and histochemical approach. **Aquaculture** 263(1–4): 259–279.

Santhanam, R. 2015. **Nutritional freshwater life.** CRC Press. Florida. 215 pp.

Saowakoon, H. and S. Saowakoon. 2007. **Breeding and nursing of spotted spiny eel (*Macrognathus siamensis*; Gunther, 1861).** Proceedings of the 45th Kasetsart University Annual Conference: Fisheries Section 2007: 712–721.

Sarasquete, C., J.M. González de Canales, J.A. Arellano, A. Muñoz-Cueto, L. Ribeiro and M.T. Dinis. 1996. Histochemical aspects of the yolk-sac and digestive tract of larvae of the Senegal sole, *Solea senegalensis*. **Histology and Histopathology** 11: 881–888.

Smith, S.L. 1989. **Digestive function in teleost fishes.** In: Fish nutrition (ed. J.E. Halver), pp. 331–421. Academic Press. San Diego.

Stroband, H.W.J. and A.G. Kroon. 1981. The development of the stomach in *Clarias lazera* and the intestinal absorption of protein macromolecules. **Cell and Tissue Research** 215(2): 397–415.

Thalathiah, S.B., A.B.O. Ahmad and B.S. Mohamad Zaini. 1988. Induced spawning of Asian fishes induced spawning techniques practised at Batu Berendam, Melaka, Malaysia. **Aquaculture** 74(1): 23–33.

Thilsted, S.H. 2010. **The potential of nutrition-rich small fish species in aquaculture to improve human nutrition and health.** Proceedings of the Global Conference on Aquaculture 2010: 57–73.

Verreth, J.A.J., E. Torreele, E. Spazier, A. Van der Sluiszen, J.H.W.M. Rombout, R. Booms and H. Segner. 1992. The development of a functional digestive system in the African catfish *Clarias gariepinus* (Burchell). **Journal of the World Aquaculture Society** 23(4): 286–298.

Walford, J. and T.J. Lam. 1993. Development of digestive tract and proteolytic enzyme activity in seabass (*Lates calcarifer*) larvae and juveniles. **Aquaculture** 109(2): 187–205.

Zaiss, M.M., I.E. Papadakis, E. Maingot, P. Divanach and C.C. Mylonas. 2006. Ontogeny of the digestive tract in shi drum (*Umbrina cirrosa* L.) reared using the mesocosm larval rearing system. **Aquaculture** 260(1): 357–368.

Zambonino-Infante, J.L., E. Gisbert, C. Sarasquete, I. Navarro, J. Gutiérrez and C.L. Cahu. 2008. **Ontogeny and physiology of the digestive system of marine fish larvae.** In: Feeding and digestive function of fishes (ed. J.E.O. Cyrino), pp. 281–348. Science Publishers, USA.