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Ontogeny of digestive organs during early developmental stages of the tropical whitespotted bamboo shark, *Chiloscyllium plagiosum* – a histological study

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This study was undertaken to address a lack of understanding of the early developmental stages of the tropical elasmobranch whitespotted bamboo shark, *Chiloscyllium plagiosum*, to assist with advancement of its aquaculture. It is the first report of the histological and ultrastructural characteristics of its developing digestive tract. The study was carried out from hatching to 60 days after hatching (DAH). Embryos for this study were maintained in the laboratory. Histological investigations yielded the following data. At hatching, the gut of an embryo consisted of an undifferentiated straight tube that lay over the yolk sac. The digestive tract was differentiated into buccopharynx, esophagus, stomach, and intestine by 8 DAH. During the preflexion phase (within 16 DAH), the mucous cells of the esophagus were differentiated. Gastric glands were present at 60 DAH, indicating the transition from the embryonic to the juvenile stage and the acquisition of an adult mode of digestion. The lumen of the intestine appeared on 6 DAH; the intestinal valve appeared and divided the intestine into anterior and posterior sections on 20 DAH. The liver and the pancreas were formed on 8 DAH. The yolk sac was partially depleted by 80 DAH. In terms of ultrastructure, on 30 DAH, the anterior section of the esophagus was lined by a stratified epithelium, whereas the posterior section was lined by simple tall columnar epithelial cells. Goblet cells and oxynticopeptic cells were found in the stomach. The pancreatic acinar cells were strongly elongated and of two types: one with a pale and the other with a more opaque cytoplasm.

Keywords: bamboo shark; *Chiloscyllium plagiosum* ontogeny; digestive organs; early developmental stages

Introduction

The whitespotted bamboo shark, *Chiloscyllium plagiosum* is the subject of this study. It is a small, common, reef-dwelling benthic shark, with a length of about 83 cm. The species is abundant throughout continental Southeast Asia and is commonly taken by inshore fisheries in China, India, Taiwan, Thailand, and Hong Kong for human consumption (Cornish et al. 2007). The sharks are usually caught by commercial

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longline or lobster trap net fisheries. Due to the continuous population decline resulting from over-fishing and habitat destruction, and the slow population recruitment because of slow reproductive and growth rates, *C. plagiosum* was reported to be a species with aquaculture potential (Xie et al. 2009; Chen et al. 2011).

Rearing embryos is considered a major bottleneck for the successful propagation of any new species, (Mai et al. 2005; Gisbert et al. 2008; Rotenstein et al. 2009; Shinsuke et al. 2010). During this embryonic period, the transition from endogenous to exogenous feeding and weaning to artificial diets are characterized by high mortality rates, often linked to an inadequate food composition. The underlying reason is that, at this time, the digestive tract is largely undifferentiated, with low digestive enzyme activity, and its short length results in rapid evacuation of ingested food. Successful development of the digestive system is crucial for survival and growth in fish embryos, because an efficient digestive system enables fish to capture, ingest, digest, and absorb food (Kjorsvik et al. 2004). Early ontogeny can be a period of rapid morphological development that provides an opportunity to study substantial changes in feeding behavior and morphology. Although larval fish may be morphologically capable of capturing food items at first feeding (Sucré et al. 2009), the digestive system needs a series of developmental changes before it becomes fully functional (Elbal et al. 2004). Various studies have therefore been undertaken in recent years to investigate the digestive system of osteichthian larval fish (Wegner et al. 2009; Pradhan et al. 2012). In contrast to osteichthian fishes, the elasmobranch feeding mechanism comprises relatively few structural elements but little information exists about the elasmobranch digestive system.

Despite the potential of *C. plagiosum* for the aquaculture industry, only a few studies on its biology have been carried out, focused mainly on its reproduction. A detailed understanding of the development of the digestive system during the embryonic stages in *C. plagiosum* reared in the laboratory is required. We centered our study on this species exclusively to document morphological detail during elasmobranch development that might help to identify limiting factors during the rearing of their embryos, thus reducing bottlenecks in the weaning process and synchronizing the stages of development with rearing technology and feeding practices.

Materials and methods

Histological analysis

C. plagiosum were maintained in flow-through aquaria at 28 ± 0.5 °C under ambient light conditions and were not fed throughout the experimental period. The salinity of the inlet water was 26.2–28.8‰. Samples of 80 embryos were collected on a daily basis from hatching to the 10th day after hatching (DAH), every 2 days from 11 to 50 DAH, and every 5 days from 50 to 80 DAH. These samples were fixed in Bouin's fluid (Scy-Tek Laboratories, West Logan, UT, USA). Ten fixed specimens from each sampling day were dehydrated in a graded ethanol series and individually embedded in paraffin. Sagittal and/or transverse histological sections (thickness: 5–7 μm) of whole specimens were stained with Haematoxylin-eosin (H-E). The sections were examined and photographed with a light microscope (Leica DM4500B).

Ultrastructural analysis

Pre-fixed samples from 30 DAH were post-fixed in 2% OsO_4 with 0.1 M phosphate buffer for 2 h, and then dehydrated and embedded in epoxy resin and SPI-812,

respectively. Ultrathin sections obtained with a Leica UC6 ultramicrotome were stained with uranyl acetate and subsequently with lead citrate. The observations and recording of images were performed with a JEM 100CX II transmission electron microscope.

Results

On 1 DAH, the total length of individual larvae was 1.50 mm. At 30 DAH, the size ranged from 45.49 to 53.11 mm in total length (mean: 50.21 mm). At 60 DAH, the animals ranged from 115.30 to 135.00 mm in total length (mean: 120.10 mm).

At hatching, the alimentary canal appeared as a histologically undifferentiated straight tube lying dorsally to the yolk sac. *C. plagiosum* underwent developmental changes leading to the differentiation of the digestive tract into four segments, namely buccopharynx, esophagus, stomach, and intestine. Within 15 DAH, the digestive tract had opened completely from the mouth to the anus (Figure 1). Further histological and ultrastructural analyses related to the differentiations of the digestive system after hatching are described below.

Yolk sac

At hatching, embryos had two large yolk sacs, an external and an internal one. The yolk sac contained several peripheral oil globules and exhibited a homogenous, acidophilic yolk. There were many blood vessels around the yolk (Figure 2(A)). This structure was surrounded by squamous epithelium. The yolk was resorbed gradually, and most of the yolk sac had not disappeared until 65 DAH, i.e. at the juvenile stage. Up to 80 DAH, the external and internal yolk sacs were small and still visible in the resorbed yolk sac (Figure 2(B)). The yolk sac matrix was also rich in proteins.

Esophagus

From the opening of the mouth at 3 DAH, the buccopharynx communicated with the anterior intestine through a short esophagus with a relatively narrow lumen (Figure 3(A)).

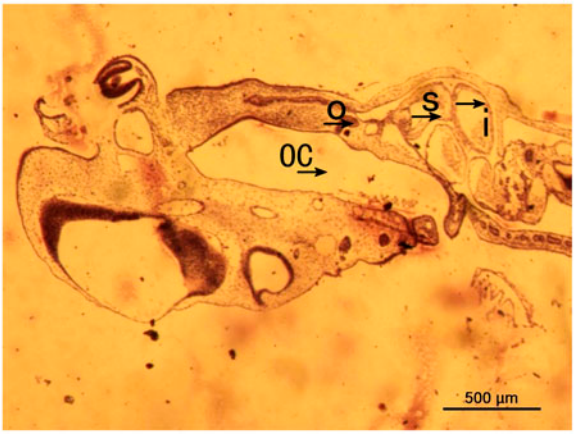

Figure 1. (Colour online) General view of the gastro-intestinal tract in a 15 DAH *C. plagiosum* embryo, showing differentiation of oral cavity (oc) → esophagus (o) → future stomach (s).

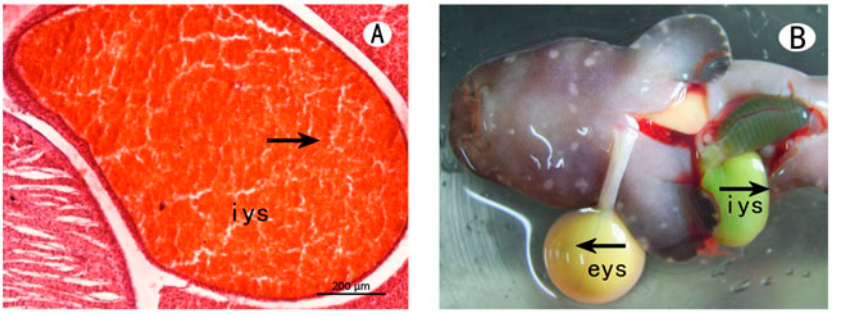

Figure 2. (Colour online) (A) Sagittal section through a portion of a 35 DAH embryo, showing internal yolk sac (→); (B) A 60 DAH juvenile showing external (←) and internal yolk sacs (→). Notes: Abbreviations: eys: external yolk sac; iys: internal yolk sac.

The inner mucosal surface of the anterior esophagus expanded into several longitudinal folds, starting from 15 DAH (Figure 3(B)). The esophagus walls consisted of a submucosa formed by loose connective tissue, a circular layer of striated muscles, and a thin outer serosa. The muscle layer became thicker as the embryos grew. A distinct muscularis was observed by 35 DAH (Figure 3(C)). A slight increase in epithelial folding and a marked increase in goblet cell density were also seen by 35 DAH. At 80 DAH, a thicker and well-developed muscularis composed of circular muscle was distinctly visible.

The ultrastructural features of the cells of the anterior section of the esophagus mucosa on 30 DAH embryos were also different from the posterior section: The anterior section of esophagus was lined by a stratified epithelium consisting of two or three layers of flattened cells that had an irregular contour and an oval or flattened euchromatinic nucleus, with a somewhat irregular outline; the scattered goblet cells were full of mucous droplets (Figure 3(D)). By contrast, the posterior section was lined by simple tall columnar epithelial cells full of mucous droplets in the apical region (Figure 3(E)).

Stomach

On 4 DAH (Figure 3(A)), the stratified epithelium of the esophagus was replaced by a monostratified, short columnar epithelium at the entrance to the stomach and a high columnar epithelium in the more caudal zone, which appeared indistinguishable from the cells of the rest of the digestive tract. On 10 DAH, the stomach exhibited a pouched shape and its epithelium began to differentiate. Three gastric regions could be distinguished: cardia, fundus, and pylorus. The first was lined by a simple cuboidal epithelium, the latter two by a thicker columnar epithelium (Figure 4(A)). By 40 DAH, epithelial folding was pronounced in the main region; a muscularis mucosa, a submucosa and a muscle tunica could be clearly distinguished in the stomach, which was surrounded by an outer serous membrane. The musculature surrounding the pyloric sphincter was well developed (Figure 4(B)). By 60 DAH, the gastric glands were distributed both dorsally and ventrally and the muscularis consisted of circular muscle (Figure 4(C)). These tubular glands were composed of just one type of secretory cell and they were devoid of microvilli on their apical border; their base was lined with a

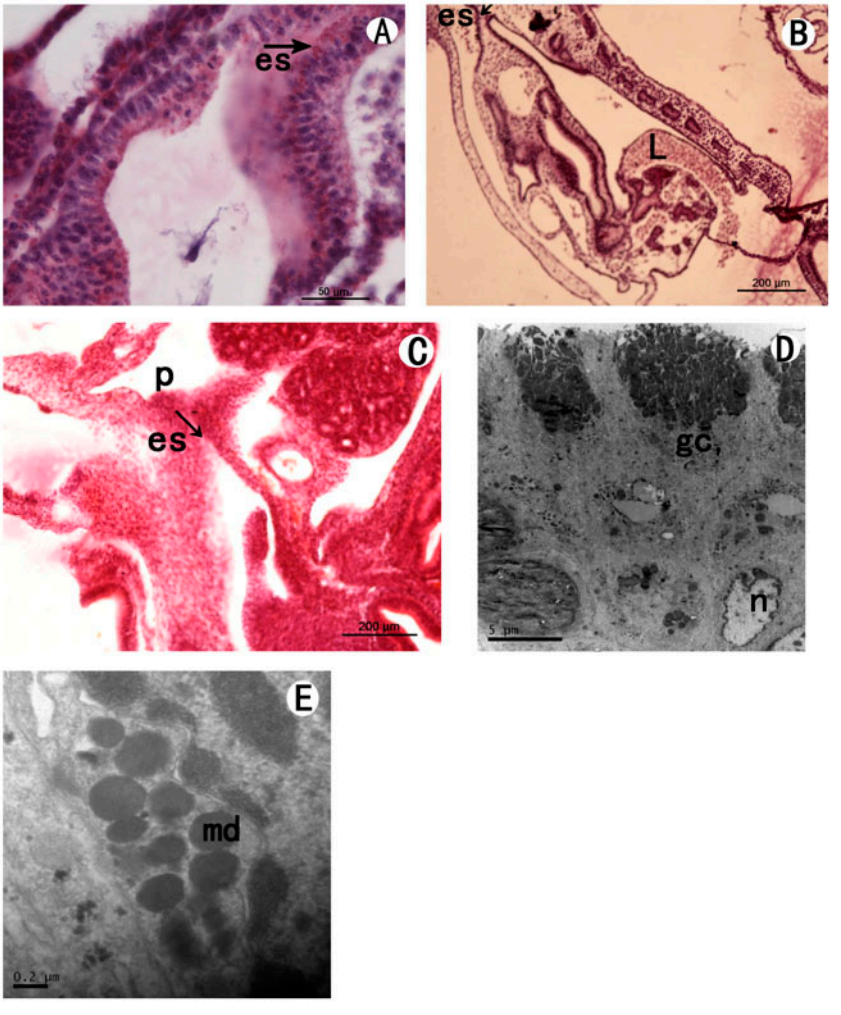

Figure 3. (Colour online) (A) Sagittal section through a portion of a 4 DAH embryo, showing the esophagus (es) (→); (B) Sagittal section through a portion of a 15 DAH embryo, showing the esophagus (es) (↙) and liver (L); (C) Sagittal section through a portion of a 35 DAH embryo, showing the esophagus (es) (↘), pharynx (p) and liver (L); (D, E) Esophagus of a 30 DAH embryo; transmission electron microscopy (TEM).
Notes: Abbreviations: gc: goblet cell; md: mucous droplet; n: nucleus.

simple cuboidal epithelium. Gastric glands were only located in the cardiac portion. The stomach wall was composed of mucosa, lamina propria-submucosa, muscularis and serosa layers. The deep mucosal layer was highly vascularized. The tunica muscularis consisted of two smooth muscle layers: an inner circular and an outer longitudinal layer. The muscular layer was thin in the cardiac portion but became thicker in the pyloric portion.

In the ultrastructure, in 30 DAH embryos, goblet cells and oxynticopeptic cells were found (Figure 4(D)). Mucous granules in the apical cytoplasm were also present. Pepsinogen granules were more abundant than in the previous phase, and the tubule

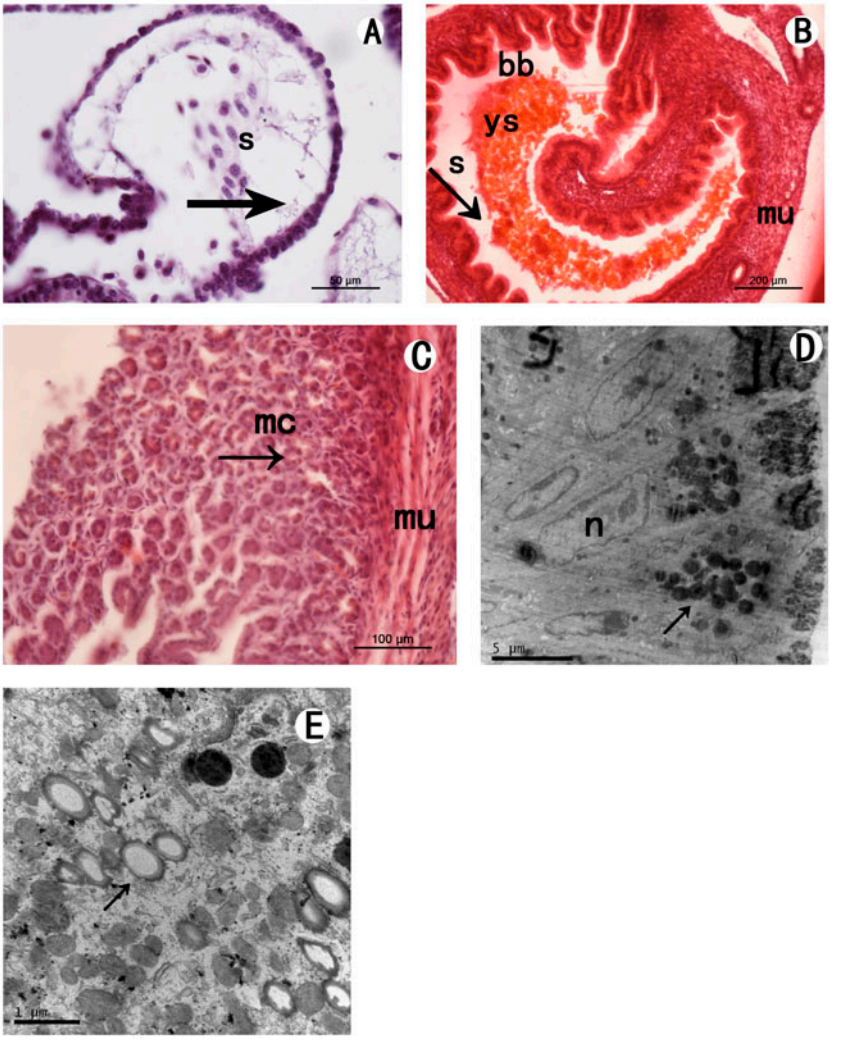

Figure 4. (Colour online) (A) Sagittal section of the developing stomach (→) of *C. plagiosum* at 10 DAH; (B) Differentiation of the stomach (s) (↘) in a 40 DAH embryo into a cardiac portion, lined by a simple cuboidal epithelium and showing the brush border of the gastric epithelium and the yolk sac; note epithelial folds separating the two portions; (C) Detail of gastric glands in the fundic stomach (→) of *C. plagiosum* at 60 DAH; (D) Oxynticopeptic cell of a 30 DAH embryo, showing the abundant tubulovesicular system; (E) Acinar gastric gland of a 60 DAH embryo.
Notes: Abbreviations: mc: mucous cells; bb:brush border; mu: muscularis; s: stomach; ys: yolk sac; n: nucleus.

and vesicle systems were not clearly recognizable. There were mucous neck cells filled with mucous granules in the apical area, next to the oxynticopeptic cells (Figure 4(E)).

Intestine

The incipient intestine appeared as a straight translucent tubular segment lying dorsally to the yolk sac. The lumen of the intestine appeared on 6 DAH (Figure 5(A)). It was

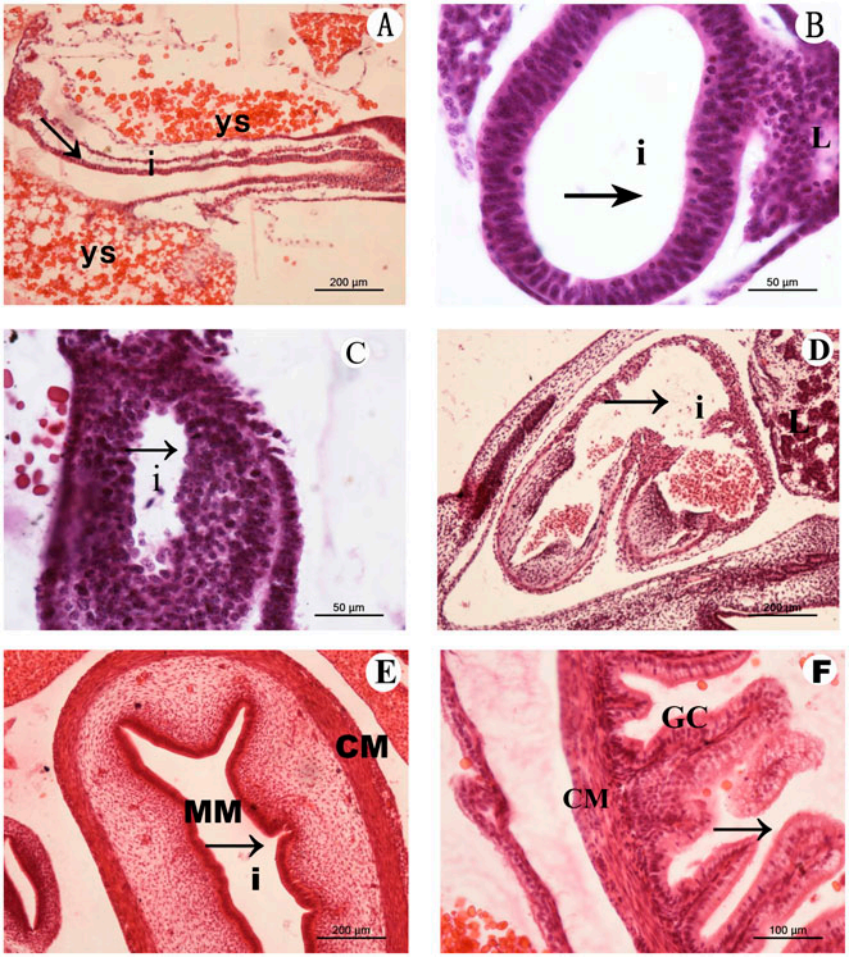

Figure 5. (Colour online) (A) Sagittal section of the intestine (↘) of a *C. plagiosum* embryo at 6 DAH. Note the nearly straight gut lying dorsally to the yolk sac, and the future intestinal valve. (B) Intestine (→) of an embryo at 8 DAH. (C) Transverse section of the anterior intestine (→) of an embryo at 11 DAH. (D) Intestine (→) of a 20 DAH embryo. Note the folding of the intestinal epithelium that occupies most of the intestinal lumen. (E) Intestine (→) of an embryos at 40 DAH, showing the folds in the mucous membrane. (F) 50 DAH, detail of folding of the intestinal (→) epithelium with scattered goblet cells. (G) Transmission electron micrograph of the intestinal epithelium of a *C. plagiosum* embryo at 30 DAH, showing large mitochondria, nucleus and microvilli. (H) Absorptive cell of the posterior intestinal epithelium in a 30 DAH embryo.- Notes: Note the lack of a terminal web, the presence of pinocytotic invaginations, and protein inclusion bodies. L: liver; GC: goblet cell; CM: circular muscle; MM: mucous membrane fold; i: intestine; ys: yolk sac; md: mucous droplet; n: nucleus.

narrow with a tendency to widen at the posterior end. The mucosa, which lacked folds and goblet cells, was lined by a single layer of columnar cells with basal nuclei on 8 DAH (Figure 5(B)). At 11 DAH, an intestinal loop was formed to accommodate the increasing length of the digestive tract inside the reduced abdominal cavity. At this time, the intestinal mucosa was mostly rectilinear with several short folds (Figure 5(C)).

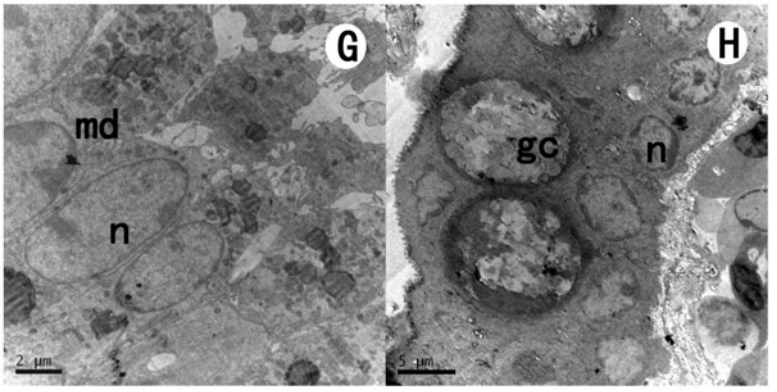

Figure 5. (*Continued*).

On 20 DAH, the goblet cells appeared in both regions of the intestine. The number of goblet cells increased with the differentiation of the intestinal mucosa, and they were more abundant in the prevalvular intestine. (Figure 5(D)). On 40 DAH, folding of the prevalvular intestinal mucosa increased and folds occupied most of the intestinal lumen (Figure 5(E)), but the postvalvular intestinal mucosa remained almost rectilinear, with very few short folds. On 50 DAH, the folds of the intestinal epithelium contained many scattered goblet cells (Figure 5(F)).

At 30 DAH, the ultrastructural analysis revealed that the mucosal epithelium of the anterior intestine consisted of tall columnar absorptive cells with a basally located nucleus, between which goblet cells were occasionally interspersed. Large electron-dense vacuoles of lipid droplets could be seen in the cytoplasm below the terminal web. Abundant mitochondria were also found in the posterior intestinal epithelium (Figure 5(G)). The picture in the posterior intestinal epithelium was, however, different. Here, the terminal web beneath the microvilli was lacking, but the presence of pinocytotic invaginations and electron-opaque protein inclusion bodies indicated that intracellular digestion was occurring by this time. There were abundant mitochondria in the cytoplasm that appeared as electron-lucent vesicles with small crista. The nucleus was oval or of irregular contour and had one or two prominent nucleoli (Figure 5(H)).

Accessory glands

Development of the liver

At hatching, the accessory digestive organ (liver) was absent and started to differentiate, on 7 DAH, from two clusters of spherical cells (Figure 6(A)). The hepatocyte cells were more clearly defined and spherical in shape on 8 DAH. The liver was located ventral to the developing gut. It appeared as a compact tissue of polyhedral hepatocytes with centrally located nuclei and reduced cytoplasm (Figure 6(B)). The liver continued to differentiate as the embryos grew, On 20 DAH, hepatocytes were arranged around hepatic sinusoids and the bile duct appeared lined with a simple cuboidal epithelium. Blood cells and liver sinusoids were also observed. The gall bladder, with a single layer of columnar cells, was located between the liver and the pancreas (Figure 6(C)). On 30 DAH, the hepatocytes became more contiguous, compared to their earlier form, and

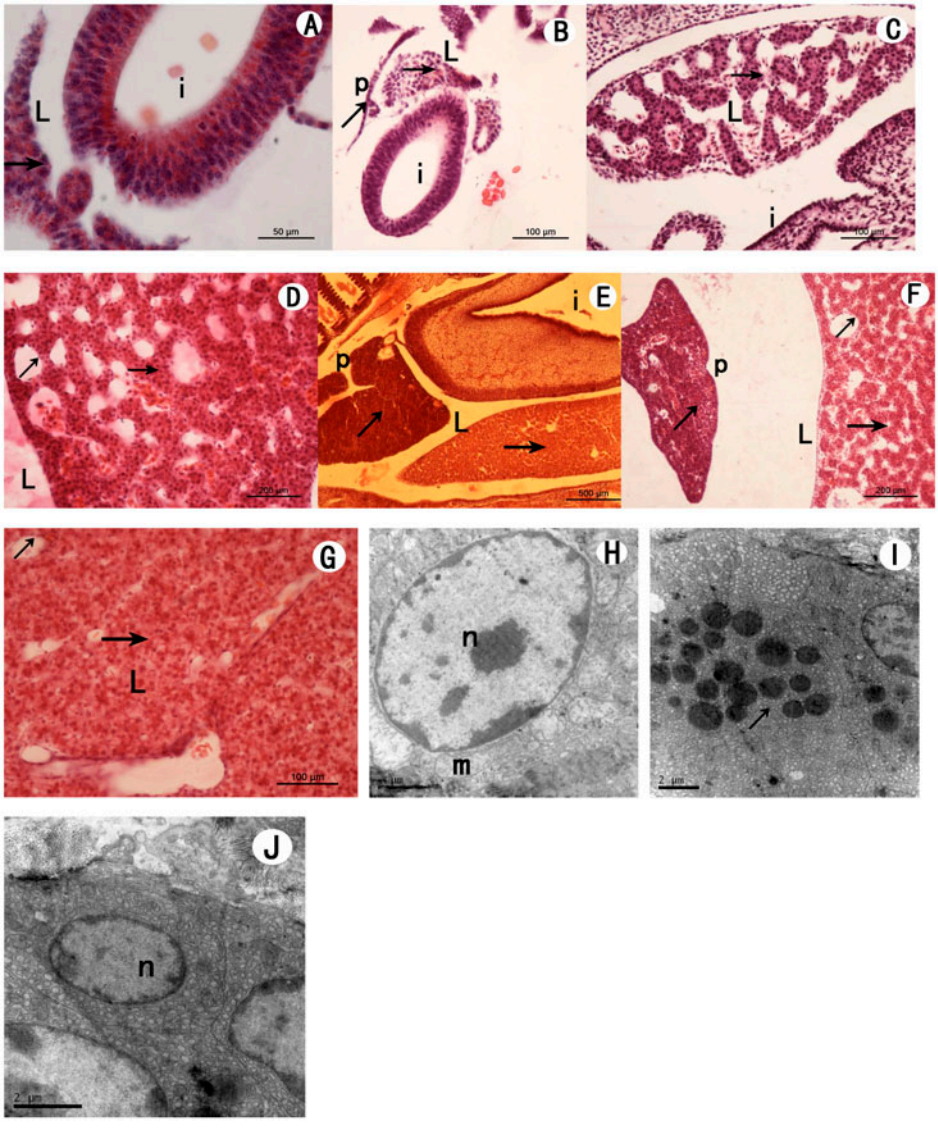

Figure 6. (Colour online) (A) Detail of the liver in a 7 DAH embryo, showing hepatocytes of the liver (→) that contain glycogen granules. (B) General view of digestive accessory organs in a 8 DAH embryo, showing liver (→) and pancreas (↗). (C) Detail of the liver in a 20 DAH embryo, showing hepatocytes (→) containing PAS-positive glycogen granules and colorless lipid vacuoles. (D) Detail of the liver in a 30 DAH embryo. Note polyhedral hepatocytes (→) with central nucleus and granular cytoplasm and colorless lipid vacuoles (arrows). (E) Differentiation of the liver (→) and pancreas (↗) in a 35 DAH embryo. Note the endocrine pancreatic cells surrounded by exocrine cells containing zymogen granules (arrows). (F) Liver (→) and pancreas (↗) at 40 DAH. Note the hepatocytes and vacuoles for lipid storage (arrows) in the liver. (G) Liver in a 60 DAH juvenile. Note the hepatocytes and vacuoles for lipid storage (arrows) in the liver (→). (H) Transmission electron micrograph of the liver cell of a *C. plagiosum* embryo at 30 DAH, showing mitochondria and nucleus. (I) Zymogene granules in pancreatic cells (arrows) of embryos at 30 DAH. (J) Development of the pancreas in a 30 DAH embryo with a more opaque cytoplasm.

Notes: Abbreviations: L: liver; P: pancreas; i: intestine; m: mitochondria; n: nucleus.

many vacuoles, for glycogen and lipid storage, were present (Figure 6(D)). The hepatocyte cytoplasm was full of lipid vacuoles by 35 DAH. The size of the lipid inclusions increased as the embryos grew, but no further major change occurred between 35 and 60 DAH (Figure 6(F) and (G)).

In the ultrastructure, in 30 DAH embryos, the liver parenchyma was richly perfused with a blood network, and the microvillar extensions into the space of disse were denser and more elongated. Cytoplasmic extensions, together with fibers of the reticular cells, formed the base for the developing stromal skeleton (Figure 6(H)).

Development of the pancreas

A cluster of basophilic pancreatic cells appeared on 8 DAH (Figure 6(C)), adjacent to the incipient intestine behind the liver. The exocrine pancreatic cells were concentrated in acini, as the pancreatic ducts appeared. The size of the pancreas, as well as the number of acini and zymogen granules in the pancreas, increased rapidly by 35 DAH (Figure 6(E)). As the embryos grew, the exocrine pancreas increased in size and contained dense eosinophilic zymogen granules (Figure 6(F)).

In the ultrastructure, in 30 DAH embryos, the pancreatic acinar cells were strongly elongated and consisted of two types: one with a pale (Figure 6(I)) and the other with a more opaque cytoplasm (Figure 6(J)). Both cell types possessed extensive rough endoplasmic reticulum, elongated mitochondria, and zymogen granules. The number of islets of Langerhans increased greatly.

Discussion

The combination of light microscopy with electron microscopy technique allowed us to present more exact data on the development of the digestive tract. The digestive tract of the *C. plagiosum* embryo was an undifferentiated tube before exogenous feeding began. The intestinal valve divided the intestine into the anterior intestine and posterior intestine by 8 DAH. Until the digestive and feeding systems develop sufficiently for prey capture and digestion, the embryos had to subsist on the maternally derived yolk sac. The degree of cross-over between the two processes can determine survival. Renwart identified an unknown luminous system in the digestive tract and photogenic organs of *Etmopterus spinax* (Renwart & Mallefet 2013). In *C. plagiosum*, the presence of a pancreas, as well as the epithelial lining of the liver and the gut suggests that the alimentary canal is equipped to assimilate food well before yolk sac absorption is completed (80 DAH).

The morphological analysis of the yolk sac structure in the juveniles of *C. plagiosum* revealed the presence of external and internal parts of the yolk. It is hypothesized that this division of the yolk is an adaptation during development that highlights both the yolk's trophoblastic layer and the liver as the distinct sites of mobilization and release of nutrients in a quantitatively significant manner. The literature on pre-implantation in a shark that possesses a yolk sac (i.e. *Rhizoprionodon terraenovae*) provides evidence of a highly organized yolk syncytium, populated by cellular organelles, and involved in systems of yolk nutrient mobilization. In this shark, droplets were released from the membrane-limited vesicles and they then fused with the membrane enveloping the yolk syncytium (Hamlett 1987). We may therefore reasonably assume a similarity between the pre-implantation structure of the yolk and utilization in the sharks and in most teleosts (Hamlett 1987). The gut of *C. plagiosum* was not even

differentiated until 40–60 DAH. From hatching until close to 80 DAH, *C. plagiosum* embryos still have a yolk sac and depend exclusively on this endogenous source of nutrition. In summary, it is assumed that its large yolk is used efficiently as the result of the additive effect of prolonged parental care.

Two differentiated regions of the *C. plagiosum* esophagus could be distinguished clearly in the histological and ultrastructural investigations of the esophageal epithelium. This is similar to reports on the Californian halibut *Paralichthys californicus* (Gisbert et al. 2004), the pandora, *Pagellus erythrinus* (Micale et al. 2006), and other oviparous elasmobranch species (Matey et al. 2009). Muco substances in the whitespotted bamboo shark that are produced by goblet cells scattered within the stratified squamous epithelium play an important lubricating role. The presence of simple tall columnar epithelium cells, which make up the posterior mucosa and are full of mucous droplets, in the apical region implied their function not only for food transportation, as in the Siberian sturgeon, *Acipenser baeri* (Gisbert & Doroshov 2003), and in *Pseudosciaena crocea* (Mai et al. 2005), but also for mucosal protection, as in *P. californicus* (Gisbert et al. 2004). Goblet cells secrete mucus to facilitate the passage of food through the esophagus and protect the mucosa against proteolytic degradation by neutralizing stomach acidity. In the present study, goblet cells in *C. plagiosum* were observed on 35 DAH in the esophagus.

In most of the embryos examined, a completely differentiated stomach appeared between 4 DAH and 35 DAH. By 15 DAH the number of goblet cells in the esophagus, stomach, and gut had increased considerably. This coincides with the appearance of the gastric glands in the stomach. Because the esophagus is so close to the stomach, the mucous secretion from the goblet cells in the esophagus may also protect the stomach from proteolytic degradation. Gisbert found goblet cells in the fundic region of the stomach in Californian halibut 30 DAH (Gisbert et al. 2004). We also found goblet cells in the stomach of *C. plagiosum* at 30 DAH.

The appearance of gastric glands marked the formation of a functional stomach, which is also a histological criterion for differentiating embryos from juveniles (He et al. 2012). For example, weaning onto dry food can be successfully achieved only after the stomach has become functional in three species of elasmobranch (*C. plagiosum*, and the little skate, *Leucoraja erinacea* (Anderson et al. 2010).

Gastric glands increase digestive efficiency, but the timing of gastric gland development varies greatly among fish species. In fast-growing species such as spotted sand bass, gastric glands were present on 16 DAH (Peña et al. 2003). Elbal found that gastric glands developed in gilthead sea bream on 59 DAH (Elbal et al. 2004). Rectal gland proteins were regulated following feeding in the dogfish shark (Dowd et al. 2008), which is 1 DAH earlier than we found in the present study. The slow organ development nevertheless indicates the slow-growing potential for metabolism in *C. plagiosum*.

The weaning of *C. plagiosum* started around 60–80 DAH, when the fundal stomach and pyloric ceacae had formed. Some studies suggest, however, that weaning should start after the appearance of the gastric glands (Lowry & Motta 2007; Jaroszewska & Dabrowski 2009; Wood et al. 2009). Our data indicate that the formation of the fundal stomach should be an important indicator for stomach function because it not only embeds gastric glands but also provides more space to hold food for a longer period to mix the food well with digestive enzymes. Similar results were found in another elasmobranch species, *L. erinacea* (Anderson et al. 2010).

In *L. erinacea*, both hydrochloric acid and pepsinogen were assumed to be secreted by one type of cell: the oxynticopeptic cells (Anderson et al. 2010). The ultrastructure

of oxynticopeptic cells in the present study indicated the asynchronous development of acid-secreting and pepsinogen-secreting functions. The appearance of gastric glands and even the appearance of pepsinogen granules in embryos of *C. plagiosum* did not correspond to the beginning of stomach function.

Our results confirm that *C. plagiosum* embryos have numerous goblet cells. Most of them were distributed on the anterior region of the esophagus. These cells play an important role in pre-gastric digestion in *Pleuronectes ferruginea* embryos. The epithelial absorptive cells of the anterior intestinal segment showed electron-opaque lipid droplets on 30 DAH.

Theodosiou demonstrated histologically distinct regions within the spiral intestine epithelium, and found subregions of the spiral intestine that account for differences in absorption in *L. erinacea* (Theodosiou & Simeone 2012). In the anterior part of the *L. erinacea* gut, few lipid vacuoles were observed in those embryos fed live rotifers. This means that there is sufficient lipid transport ability in the intestine of *C. plagiosum*.

The liver and pancreas in *C. plagiosum* differentiate early in development, as in several teleost species and Chondrichthyes (Wright & Wood 2009; Comabella et al. 2012; Pradhan et al. 2012). The liver and pancreas expanded rapidly and contained vacuoles within 20 days of hatching, which indicates that they were already functional areas for lipid and glycogen storage (Claes & Mallefet 2010). The first signs of nutrient absorption were observed 65 days after exogenous feeding started. The liver volume generally increased, and the amount of rough endoplasmic reticulum and Golgi apparatus increased as the embryos developed. The accumulation of lipid vacuoles in the liver indicated the gradual functional development of the digestive tract in *C. plagiosum*. The position of the nucleus in the hepatocyte cytoplasm reflected the degree of accumulation of nutrient reserves. Similar results were found in other fish species. The digestive gland cells of marine mussels were, however, the major environmental interface for the uptake of contaminants, particularly those associated with natural particulates that are filtered from seawater by mussels (Moore & Allen 2002).

This study is the first to investigate the morphology of the digestive organs in a tropical elasmobranch. Overall, whitespotted bamboo shark embryonic development was similar to that of other oviparous elasmobranches, in terms of gross embryo characteristics and behaviors (Anderson et al. 2010). The early embryonic period of *C. plagiosum* is a period of intense development where critical structures are forming and developing, followed by a period of system refinement prior to settlement, rather than a second period of critical development. The extensive development that occurred at the transition from endogenous nutrition to exogenous feeding suggests that this is a critical period in the development of *C. plagiosum*. The alimentary canal and the stomach differentiate. Yolk sac absorption is the second period of most intense and rapid morphological development. The present study has identified a number of similarities in yolk sac absorption of *C. plagiosum*, compared to the Silver Arowana (*Osteoglossum bicirrhosum*). The digestive enzymes of *Ruditapes decussatus* and *Venerupis pullastra* juveniles could, however, be used as indicators of their nutritional condition (Albentosa & Moyano 2008).

In general, the rate at which development occurs suggests a life history style that supports the vulnerable pre-competent life stages to overcome critical periods in development and survive the benthic environment.

Disclosure statement

No potential conflict of interest was reported by the authors.

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