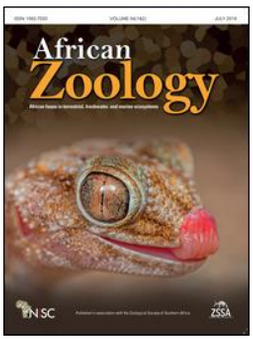

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Morphology and functional ontogeny of the digestive tract of *Barbus altianalis* larvae

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The ontogenetic development of digestive structures in Ripon barbel (*Barbus altianalis*) larvae was investigated using standard histological and histochemical procedures from hatching up to 60 days after hatching (DAH). The study was conducted to determine the best period of exogenous feeding and the stage when the digestive tract is able to digest processed microdiets. Results indicated that at hatching, the digestive tract, mouth and anus were closed. The opening of the mouth and anus were observed 3–4 DAH, whereas complete separation of the entire gut was observed on 5 DAH. Exogenous feeding started 5–6 DAH, but complete yolk exhaustion occurred 7–8 DAH, indicating a period of mixed feeding. Mucosal epithelial folds were first noted 3 DAH in the anterior intestine and became profound with some goblet cells (mucous cells) by 6 DAH. At 7 DAH the mucous cells had started secreting both neutral and acid glycoconjugates. The first intestinal single loop occurred at 28–30 DAH and a double loop at 45–50 DAH. Each coiling was proceeded by larval weight increase. By 7 DAH the buccopharyngeal cavity was lined by a layer of squamous epithelial cells with scattered goblet cells and tastebuds that became numerous by 15 DAH. At hatching, the liver and the pancreas were undifferentiated, but on 3 DAH the hepatocytes and zymogen granules of the pancreas became clear. By 7 DAH both organs enlarged, making extensions into the posterior. Intestines coiling at 28–30 DAH coincided with the beginning of external dressing of the scales, a period when *B. altianalis* started transforming into a juvenile. By 7–8 DAH the digestive structure showed all the necessary digestive features that could enable the larvae to digest any compound diet suggesting that it may be feasible to substitute or offer a complete microdiet during larvae nursing with reduced larval mortality.

Keywords: digestive structures, exogenous feeding, microdiet, mucins, ontogenetic development, Ripon barbel

Introduction

The Nile Ripon barbel, *Barbus altianalis*, is a newly domesticated cyprinid of high commercial value, cherished by many communities in Uganda and other East African countries. It is distributed in Lakes Victoria, Edward and George including all the rivers associated with these lakes (Snoeks et al. 2012; Chemoiwa et al. 2013). However, larval survival rates in culture systems are still very low, which hinders its massive seed production potential for commercial aquaculture. One of several aspects in fish culture for mass seed production entails reducing larval mortalities and increasing larval growth rates, but this requires clear knowledge of morphological and functional structure of the digestive system of the developing larvae. Understanding larval ontogenetic development is crucial for larval fish rearing in any economically important aquaculture species (Chen et al. 2006), in part because introducing a correct feed type at a particular larval developmental stage is a key challenge in intensive fish culture (Kolkovski 2001; Ostaszewska et al. 2003).

The assessment of larval development has been studied in many cultured fish species to determine the period when the digestive system is ready to breakdown and assimilate artificial feeds (Sarasquete et al. 1995; Cahu and Zambonino 2001; Zambonino-Ifante et al. 2008). The developmental stage of the digestive structure determines the timing and defines the protocol for larval weaning. How soon the larvae will feed and get weaned to a microdiet will depend on the functional development of the digestive system. In some fish species, including ide, *Leuciscus idus*, another cyprinid, the digestive system is well advanced with functional enzymes which may allow digestion of microdiets at the time of first exogenous feeding (Ribeiro et al. 1999; Ostaszewska et al. 2003). In other fish species, including common carp, *Cyprinus carpio* the digestive system is not properly developed to digest introduced feed particles at first feeding (Sarasquete et al. 1995; Hamlin et al. 2000; Garcia-Hernandez et al. 2001). First feeding, a period when exogenous feeding begins, is considered the most critical period as delays or early feeding may affect larval growth and survival (Kamler 1992; Mai et al. 2005). Even though most fish may develop through the same basic phases, the rate and complexity of development of the digestive tract may differ as the larvae matures, indicating varying nutritional requirements at different stages and periods in

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different fish species (De Silva and Anderson 1995; Chen et al. 2006; Zambonino-Ifante et al. 2008).

In *B. altianalis*, it was morphologically observed that the yolk was only visible within about 2–3 days after hatching (DAH) and feeding started 3 DAH (Rutaisire et al. 2015). The newly hatched larvae were fed on Raanan feed (56 Crude Protein CP%, made by Raan Feed Company from Akko City in Israel), but high larval mortality and slow growth were noted. Although there are other factors that could contribute to larval mortality, it is essential that the ontogenetic development in *B. altianalis* is studied and understood. Knowledge of ontogeny of the digestive structure will contribute to the understanding of larval physiology and determine the appropriate period of first or mixed feeding, acceptability of compound diets as well as the nutritional requirements at each developmental stage for effective development of weaning technology of *B. altianalis* in hatcheries. The aim of this study was to describe the ontogenetic development of the digestive system from hatching until metamorphosis with the view of understanding the organisation and functionality of the system, to provide the basis for developing nutritional requirements, and to identify the period for introducing compound feed to the larvae.

Materials and methods

Fish larvae, sampling and external morphological observations

Barbus altianalis broodstock with ripe eggs were obtained from the Victoria Nile at Jinja (0°35′00′ N, 33°05′00′ E) and transferred in aerated 250–litre water tanks to the Aquaculture Research and Development Center (ARDC) Kajjansi (0°36′25′ S, 32°68′00′ E) where they were facilitated to spawn using water flow (Rutaisire et al. 2015). The current study was conducted between November and December 2016. Eggs were incubated in three hatching tanks of 50 litres at 27 °C and hatched after 68.00 ± 0.88 hrs (77 degree-days). Immediately after hatching they were transferred and randomly assigned to three glass tanks for better management and growth. The hatching day was considered as Day 0. The larvae started feeding at 5–6 DAH on decapsulated *Artemia* until 30 DAH. A commercial dry feed (57% Crude Protein CP) was gradually introduced for four days until the amount of *Artemia* was reduced to half the original quantity. Feeding with both *Artemia* and dry feed continued for 15 more days until 45 DAH when the decapsulated *Artemia* was gradually and completely removed in a transitional period of four days. The larvae were kept on dry (microdiet) feed alone until 60 DAH. Feeding was done to satiation.

In order to examine and describe the histology of gut structure of larvae, a sample of 10 larvae was randomly drawn from the three tanks. They were anaesthetised with AQUI-S (manufactured by AQUI-S New Zealand Ltd), weighed and preserved in Bouin's solution, for more than 45 hrs before being processed following standard histological procedures by Bancroft and Gamble (2002). The samples were preserved daily from day 0 for the first 10 days, then every two days until 20 DAH, every three days up 30 DAH, every 5 days from 30 DAH up to 60 DAH. In every subsampling, another 3 larvae were taken from each of the 3 tanks, anaesthetised with AQUI-S and were examined under the light microscope (Model Leica DM 500, made by Microsystems Switzerland Ltd) to observe any changes in the gut development that would be correlated with the preserved samples. Because the young larvae were very transparent, the internal gut structures were easily observed under the light microscope until 20 DAH, when the gut became opaque. The gut structures examined were the lips, mouth, buccal cavity, pharynx, oesophagus, intestines, liver, pancreas and the anus from day 0–60 DAH.

To estimate the Specific Growth Rate (SGR) accurately with adequate sampling, about 40 larvae were sampled from each tank, weighed after every 15 days before being returned to the tanks. All the larvae were weighed to the nearest 0.001 g and their lengths recorded to the nearest 1.0 mm.

To avoid accumulation of ammonia in tanks, tanks were cleaned at 7.00 h and 17.00 h daily with replacement of two-thirds of the water in the tanks after every cleaning. The ammonia levels were maintained at 0.1 ± 0.05 ppm using LaMotte Fresh water aquaculture kit (Code 6665-02-CC). Temperature was maintained at 27 ± 1 °C using thermostatic heating rods (Sera Aquarium heater thermostat; sera D 52518, Heinsberg Germany).

Histological and histochemical procedure

Small larval tissues of 5–7 µm size were sliced at different points along the gut structure of the preserved larvae. Tissues were taken at the same points for each larva. They were processed following standard histological methods by Bancroft and Gamble (2002). They were stained using Gill's haematoxylin and eosin (H&E). To determine the presence of neutral and acidic glycoconjugates (mucins), some sections were stained with periodic acid-Schiff/PAS and Alcian blue/AB (pH 1 and 2.5) and were processed following the procedure by Pearse (1985). Both longitudinal and cross tissue sections were examined using a Carl Zeiss light microscope (model Leica DM 500) at different magnifications. Photomicrographs were taken using a Canon PowerShot A640 10 megapixels camera mounted onto the microscope to aid with description of the digestive tract of the fish.

Data analysis

The larval growth was expressed as variation of mean weight (w) with culture period and as specific growth rates (SGR), i.e. percentage per daily increment (Hopkins 1992; Chen 2006). Specific growth rate was calculated according to the equation:

$$SGR = [\ln(Fw) - \ln(Iw)]/\Delta T \times 100$$

Where ln = the natural log; Iw = initial weight; Fw = final weight; ΔT = number of culture days. The buccopharynx layer, goblet cell and tastebud sizes were measured using a calibrated stage micrometer in the eyepiece lens under a light microscope. For the goblet or mucous cells, a mean value of three measured planes was calculated for each slide from each individual larva before the sample mean was recorded. However, a single value of the length of tastebuds was measured from each larva and the mean value of the sample was recorded. Three planes were measured across the buccopharynx layer and a mean

value was recorded for each larva. The number of intestinal loops formed was counted under the microscope and recorded. The mean weight values for three replicates and the relationship between weight W and length TL were calculated by regression using Microsoft Office Excel 2010. Data are shown as means ± SD (standard deviation).

Results

Larval growth and development

At hatching, the average total weight (W) of *B. altianalis* larvae was 2.8 ± 0.63 mg, whereas the total length (TL) was 0.79 ± 0.032 cm. The mean weight (W) increased exponentially over the experimental period (Figure 1). The mean w was strongly and positively correlated with the TL and their relationship was of a power nature ($r = 0.97$; $y = 4.508 \times 10^{3.321}$). The specific growth rate SGR was 3.02%.

At hatching the body of the larva was transparent with a large oval shaped yolk sac which reduced gradually until it flattened at 7–8 DAH (W 4.5 ± 0.98 mg; TL 1.1 ± 0.02 cm) (Figures 2a, 2b and 2c). The fins were transparent and the tail was not forked. The larvae were confined and congregated together at the bottom of the hatching tank most of the time until 5–6 DAH (W 4.3 ± 1.26 mg; TL 1.05 ± 0.05 cm) when they began swimming slowly upwards in search for food. At hatching, the eyes were brown and gradually become more pigmented, turning dark by 5 DAH when the larvae began to search for food. Active swimming at 7 DAH (W 4.4 ± 0.71 mg; TL 1.11 ± 0.02 cm) and onwards was characterised by a constriction at the anterior side of the swimbladder that separated the swimbladder into two portions (Figure 2d) and coincided with division of the tail forming a 'fork'. Under the light microscope, hexagonal transparent cells (the hepatocytes) of live larvae were observed at 2 DAH and grew from just beneath the cranial position of the swimbladder and increased in size, number and length, growing towards the posterior region (Figure 2b). The digestive tract at hatching was observed to be a small short and closed tube, but by 7 DAH the epithelial invaginations forming folds could be observed. These intestinal folds grew much deeper as the animal increased in size and formed visible intestinal loops under the microscope (Figures 2f, 2g and 2h). At 28–30 DAH (W 36 ± 9.0 mg to 40.2 ± 12.77 mg; TL 1.80 ± 0.11 cm to 1.95 ± 0.16 cm). The larvae began developing scales and this process continued even after the experiment was terminated at 60 DAH (W 193.85 ± 35.24 mg; TL 2.90 ± 0.24 cm).

The digestive system

At hatching, the larvae had a large acidophilic yolk volume (H&E stain) with a thin syncytium separating it from the primordial digestive tract (Figures 2 and 3). The digestive tract did not communicate with the exterior, because both the mouth and the anus were closed. The digestive tract was separated into a buccopharyngeal cavity, the oesophagus, the intestine and the associated digestive organs (the liver and the pancreas).

The buccopharyngeal cavity and the oesophagus

At hatching the mouth was closed and it opened between

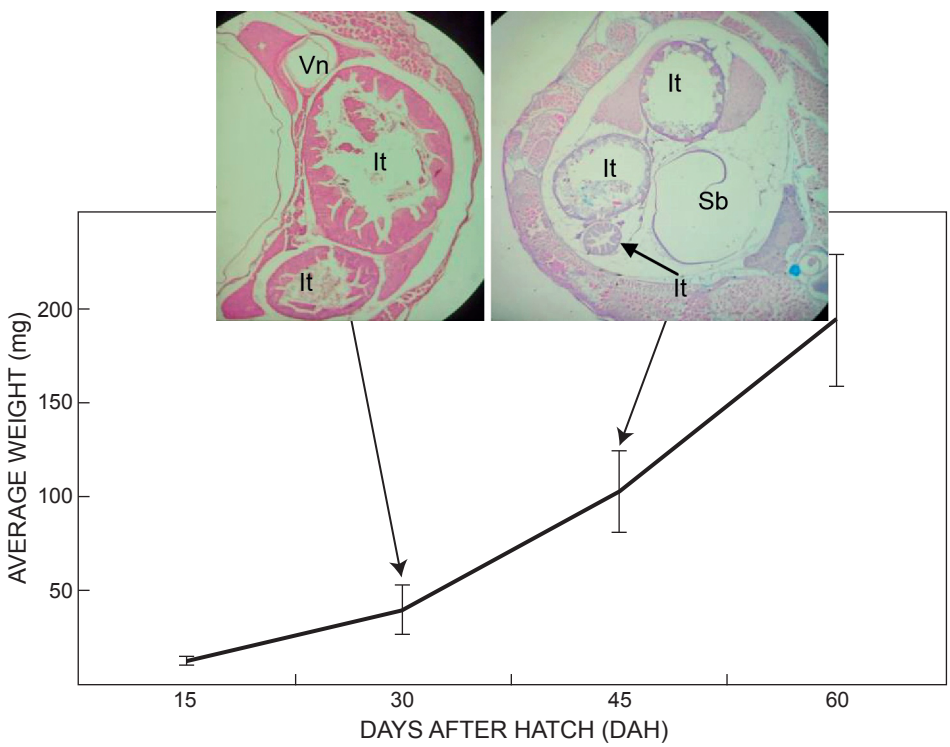

Figure 1: Variation of mean weight (mg) ($n = 3$) with days after hatching (DAH) in *B. altianalis* larvae. The micrographs show the intestinal coiling (single loop) in the upper abdomen at 30 DAH (H&E) and a double loop at 45 DAH (PAS, AB-PH 2.5). Vn, vein; It, intestine; Sb, swimbladder

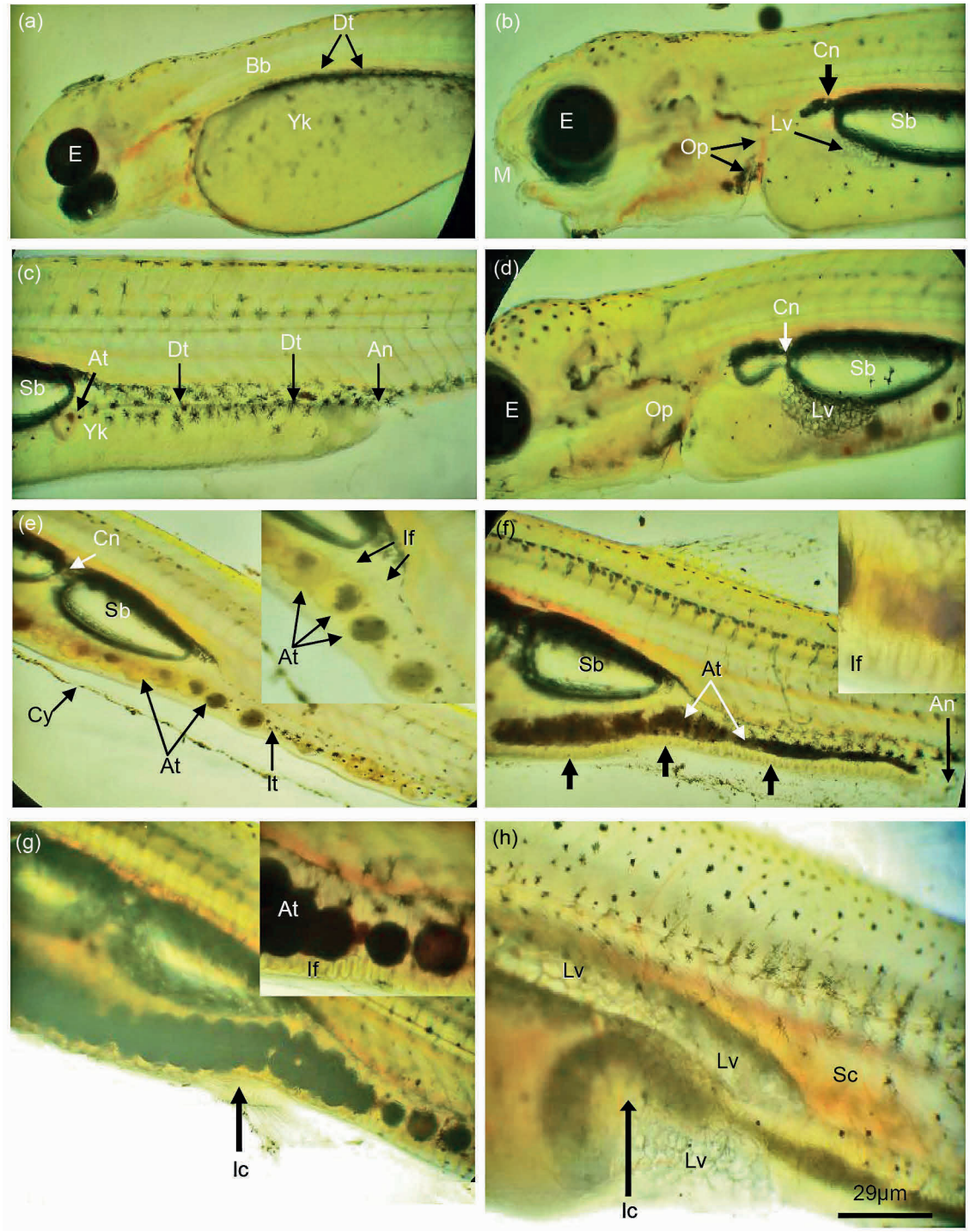

Figure 2: Gut morphology of live *B. altianalis* larvae viewed under a light microscope. (a) Larva at 1 DAH with a large yolk and an intestinal tube over the yolk. (b) Larva at 5 DAH with reduced yolk, open mouth, liver cells and a constriction which begins as an outgrowth. (c) Abdominal section of larva of figure (b) at 5 DAH, with a digestive tract containing a single *Artemia* cyst. (d) Larva at 7 DAH; there is a constriction on the swimbladder separating it into two. The liver below the swimbladder enlarges along and around the gut length toward the posterior. (e) Larva at 10 DAH; intestine with folds also defined by the increased number of *Artemia* cysts (inset are folds). (f) At 15 DAH; numerous and enlarged intestinal folds (also shown inset). (g) At 22 DAH; a bend in the intestine; intestine filled with *Artemia* cysts. (h) At 30 DAH; large liver around the intestine, intestine loop is observed. At, *Artemia*; An, anus; Bb, back bone; Dt, digestive tract; Yk, yolk; E, eye; M, mouth; Op, operculum; Lv, liver; Sb, swimbladder; Cn, constriction of the swimbladder; Cy, cyncytial layer; Sc, scaleration; Ic, intestinal coiling; It, intestine; If, intestinal folds

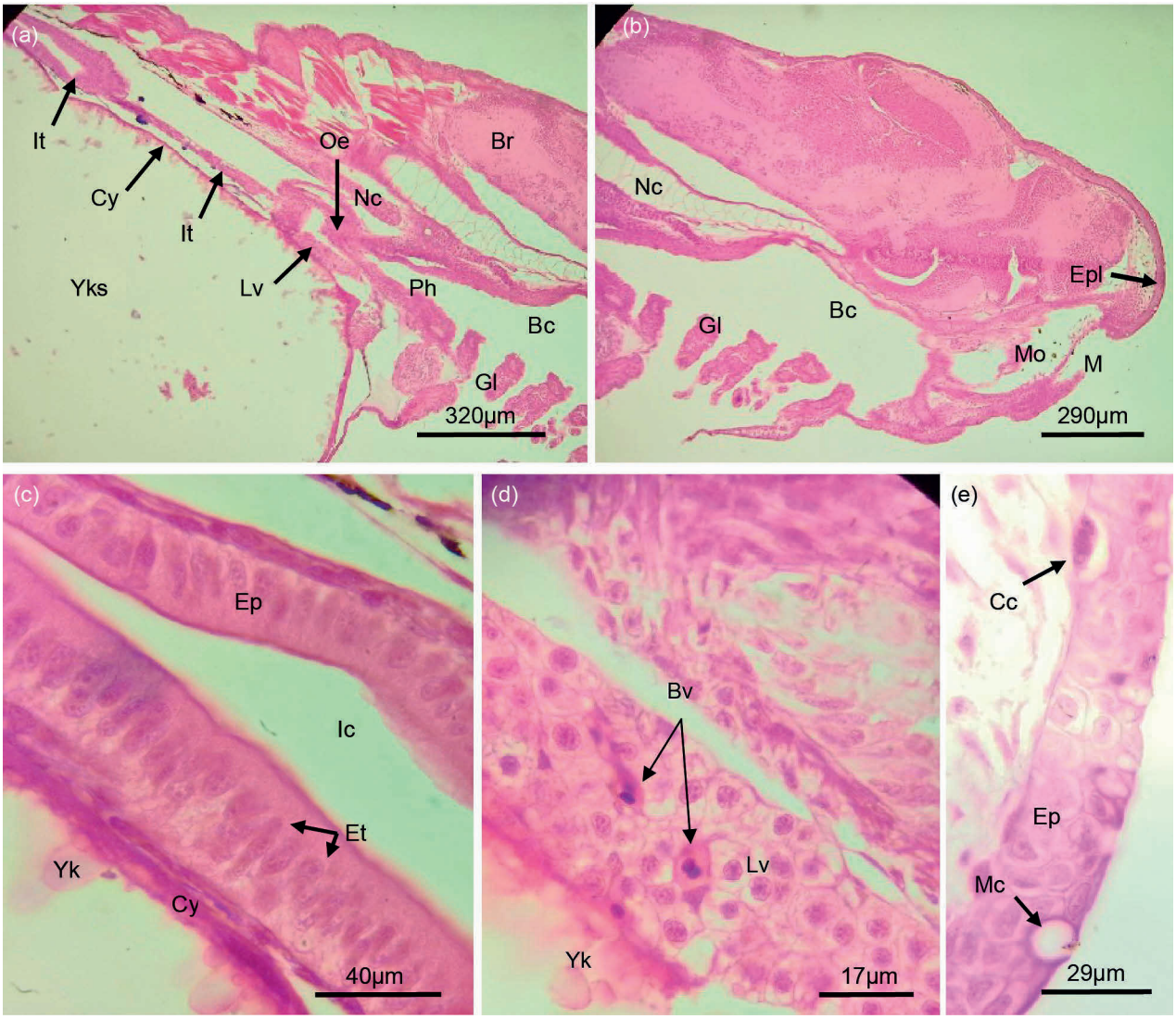

Figure 3: A section through the head and trunk of 3 DAH *B. altianalis* larvae. (a) A small intestinal tube dorsal to the yolk. (b) Buccal and mouth cavities. (c) Magnified section of (a) showing intestine. (d) Magnified section of (a) showing a portion of liver. (e) Magnified section of (b) showing epithelium around the outer lip. Oe, oesophagus; Ep, epithelium; Bc, buccal cavity; Mo, mouth cavity; Bv, blood vessels; Cc, club cells; It, intestine; Ic, intestinal cavity; Lv, liver; Cy, cyncytial layer; Nc, notochord; Et, enterocytes; Mc, mucous cells; Gl, gills; Ph, pharynx; Yks, yolk sac; Br, brain; Epl, epithelium around the outer lip; M, mouth

3 and 4 DAH (W 4.15 ± 2.5 mg; TL 0.96 ± 0.047 cm). The buccopharyngeal cavity was clearly and largely lined by a single layer of squamous epithelial cells. Intermittent with epithelial cells were tastebuds, goblet (mucous) cells and club cells that were clearly noticed by 2–3 DAH (W 4.37 ± 1.9 mg; TL 0.91 ± 0.07 cm). Between 6 and 7 DAH (W 4.29 ± 1.19 mg; TL 1.09 ± 0.038 cm), the buccopharynx had epithelium composed of stratified squamous and cuboidal shaped cells. The mucosal epithelium formed short pharyngeal folds and had several tastebuds and numerous scattered goblet cells along the buccopharynx (Table 1; Figures 4b, 5c, 5d and 5e). These goblet cells were PAS-AB (pH 2.5) positive giving a characteristic weak blue/purplish colouration. However. at 12 DAH the colour became moderate and after 15 DAH (W 12.87 ± 2.09 mg; TL 1.38 ± 0.089 cm) it was intense with numerous mucous cells (Figure 6). However, the goblet cells tested negative with AB (pH 1.0) throughout the experimental period. By 15 DAH both the goblet cells and tastebuds increased in number and size (Table 1) as the larvae grew. The tastebuds became elongated and the epithelium grew in thickness (Table 1) constituting largely pseudostratified squamous cells. The mucosal epithelial pharyngeal folds became prominent and by 45 DAH (W 102.98 ± 21.60 mg; TL 2.34 ± 0.14 cm) they become much deeper with numerous goblet cells (Figure 6c). The pharyngeal cavity at this point was characterised by a clear palatal organ with several layers of cells.

On 3 DAH the oesophagus was observed connecting the pharynx to the intestines. It was lined by a pseudostratified columnar mucosal epithelium. The epithelium had few

Table 1: The mean length of epithelial folds, goblet cells and tastebuds for various stages of development (mean ± SD)

Days after hatching	2–3	7–8	14–15	28–30	50–60
Buccopharynx layer (µm)	12 ± 2; *n* = 9	20 ± 2.1; *n* = 10	25.62 ± 10.98; *n* = 6	41.48 ± 5.2; *n* = 10	48 ± 4; *n* = 10
Nature of epithelium in buccopharynx	Simple squamous	Stratified squamous	Stratified squamous	Pseudostratified squamous	Pseudostratified squamous
Size of tastebuds (µm)	15.86 ± 1.2; *n* = 8	20.5 ± 4.5; *n* = 12	26.8 ± 2.44; *n* = 11	39.04 ± 9.76; *n* = 11	45.8 ± 8.3; *n* = 12
Goblet cells in buccal cavity (µm)	9 ± 2.3; *n* = 5	10.8 ± 2.9; *n* = 15	11.03 ± 1.9; *n* = 10	12.30 ± 2.0; *n* = 6	13.8 ± 2.9; *n* = 15
Intestinal loops (number)	Single tube	Single tube	Single tube	Double	Double
goblet cells in intestines (µm)	5.6 ± 1.1; *n* = 10	7.8 ± 2.1; *n* = 5	9.92 ± 1.2; *n* = 7	10.30 ± 2.03; *n* = 8	13.8 ± 1.22; *n* = 10

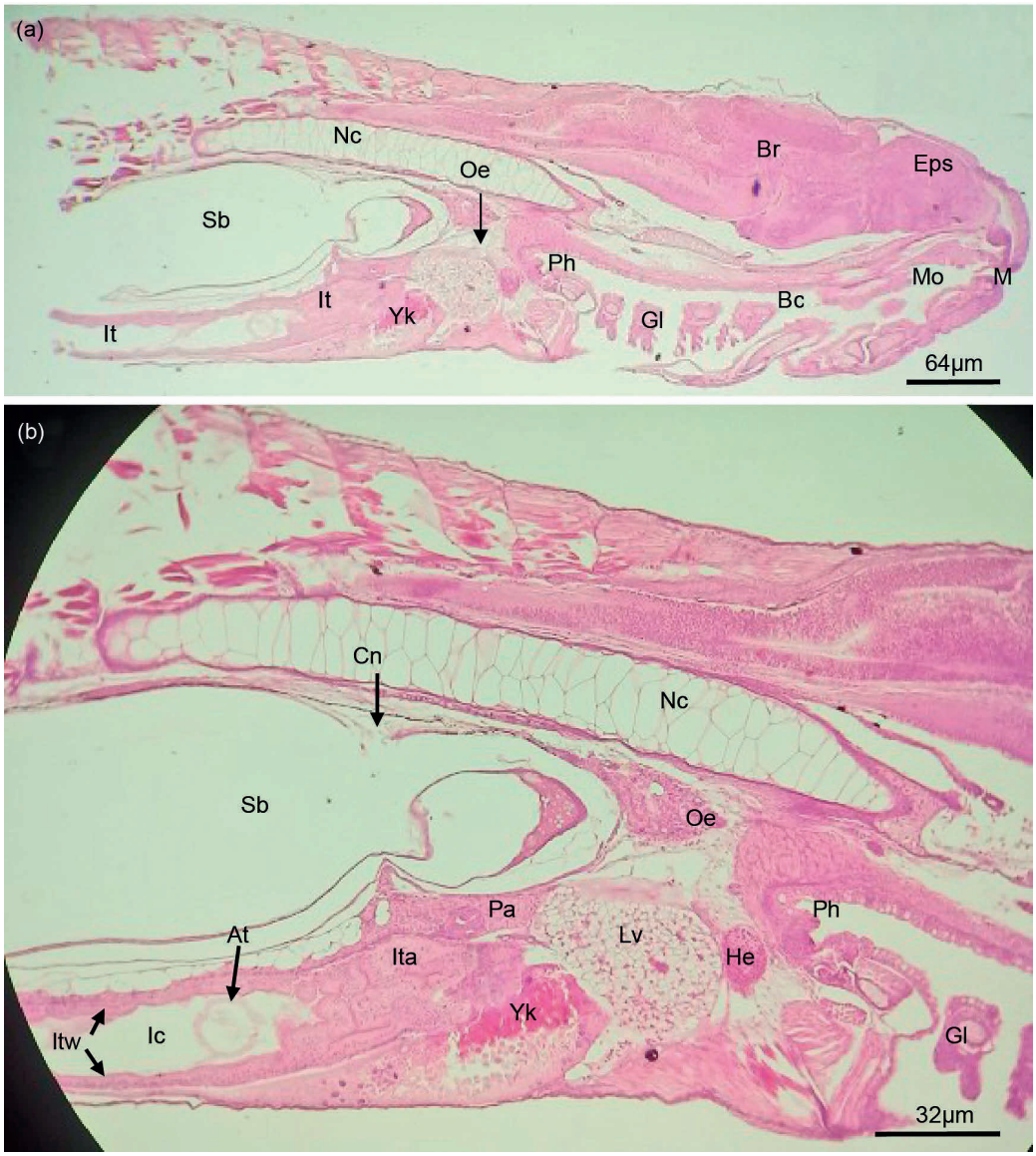

Figure 4: Longitudinal section of *B. altianalis* larva at 7 DAH: (a) Features of larva at 10 DAH, the yolk residues are still observed, anterior section is coiled. The pancreas and liver are enlarging. (b) Features of a middle section of (a) shown at a higher magnification. Itw, intestinal wall; Ita, anterior intestine; Pa, pancreas; He, heart; Eps, eye position; Oe, oesophagus; Bc, buccal cavity; Mo, mouth cavity; Sb, swimbladder; Oe, oesophagus; It, intestine; Ic, intestinal cavity; Lv, liver; At, artemia; Nc, notochord; Cn, constriction of the swimbladder; Gl, gills; Ph, pharynx; Yk, yolk; Br, brain

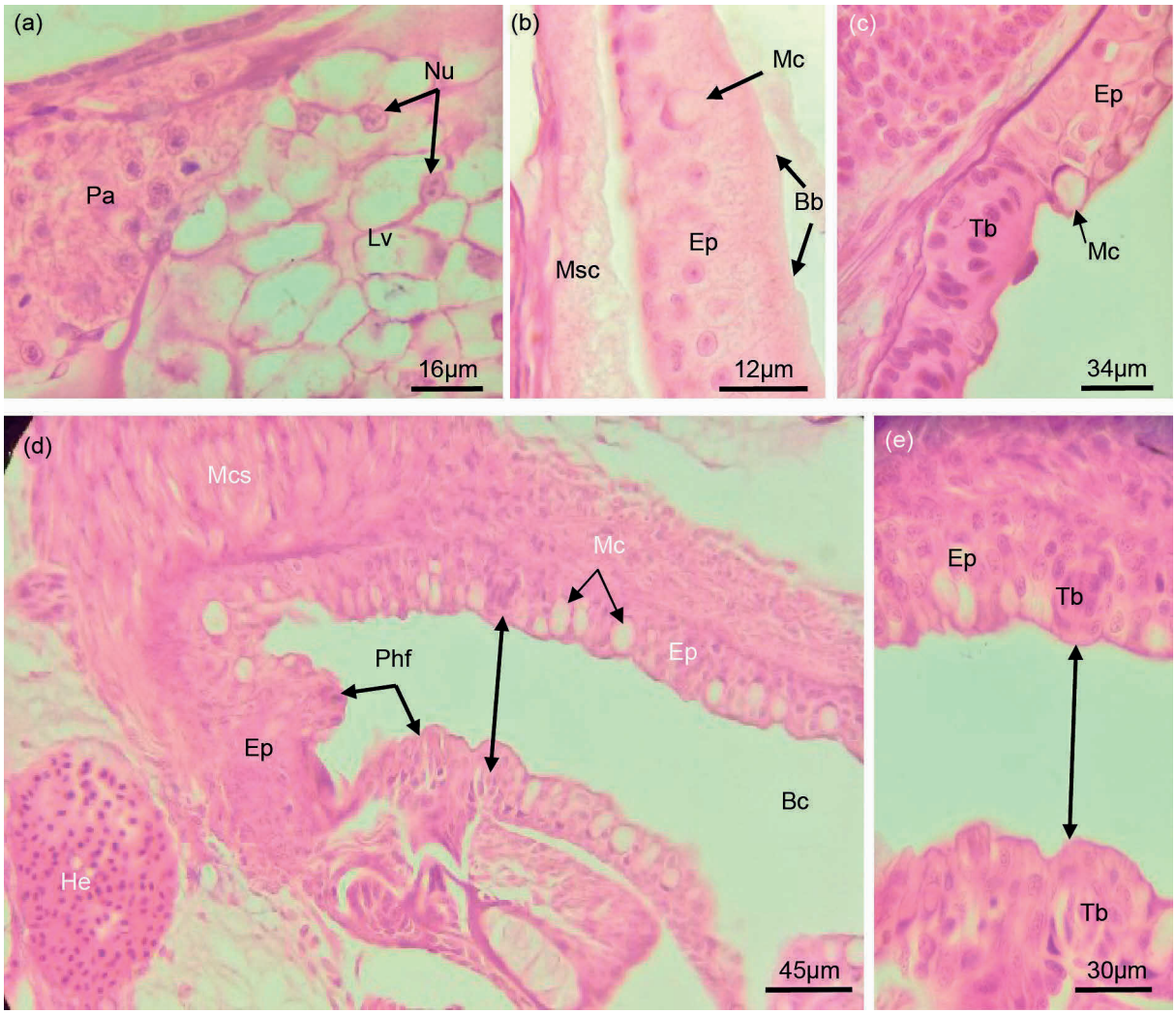

Figure 5: Histological sections of figure (4) of *B. altianalis* larva at a higher magnification. (a) Differentiated pancreas and liver at 7 DAH. (b) Mucosal epithelium of intestines showing columnar cells. (c) Buccal cavity lined with tastebuds and mucous cells. (d) Epithelium organised into pharyngeal folds. (e) Tastebuds interspaced with mucous cells in the epithelium of the pharynx. Lv, liver; Pa, pancreas; Phf, pharyngeal folds; He, heart; Nu, nucleus; Msc, muscularis layer; Tb, tastebuds; Ep, epithelium; Bb, brush border; Mc, mucous cell; Mcs, pharangeal muscles; Bc, buccal cavity

goblet cells, beneath it was a thin layer of submucosa surrounded by a thick layer of a mixture of connective tissue with smooth and striated muscles. By 7 DAH the folds were conspicuously present with increasing goblet cells. At 11 DAH (W 6.0 ± 1.15 mg; TL 1.21 ± 0.06 cm) the folds were elongated further with numerous oil globules.

The intestine

At hatching much of the digestive tract length was an intestine that lay dorsal to the yolk mass and was closed with no clear lumen observed. The intestine was lined by a mucosal epithelium consisting of a single lining of squamous and cuboidal shaped cells. The epithelium had no intestinal folds. On 1 DAH (W 4.0 ± 2.28 g; TL 0.81 ± 0.064 cm) the gut epithelial layers separated at some points forming a lumen beginning at the anterior just below the swimbladder. Subsequently on 2–3 DAH thin 'villi' (folds) were seen in the anterior (cranial) intestine. Mucosal epithelial cells at 3 DAH elongated and became columnar shaped in a single layer (Figure 3c). By 5 DAH the entire intestinal length had opened with a visible lumen. The anus and the mouth were opened between 3 and 4 DAH. The opening of the mouth and anus coincided with the reduction in the yolk size and the beginning of the constriction of the swimbladder at 5 DAH. However, commencement of feeding on decapsulated *Artemia* began at 5 DAH with about 25% of the larvae guts containing at least one or two *Artemia* cysts (Figure 2c). Feeding by the majority of the larvae (75%) occurred at 6 DAH. At commencement of exogenous feeding (6–7 DAH) the intestine was a short tube wider at the anterior with prominent folds and narrower at the posterior and its length or shape was clearly marked by the presence of decapsulated *Artemia* (Figure 2d; Figure 4).

Histological sections additionally revealed that little yolk was still observed by 7 DAH in some of the larvae (Figure 4) and complete yolk exhaustion occurred by

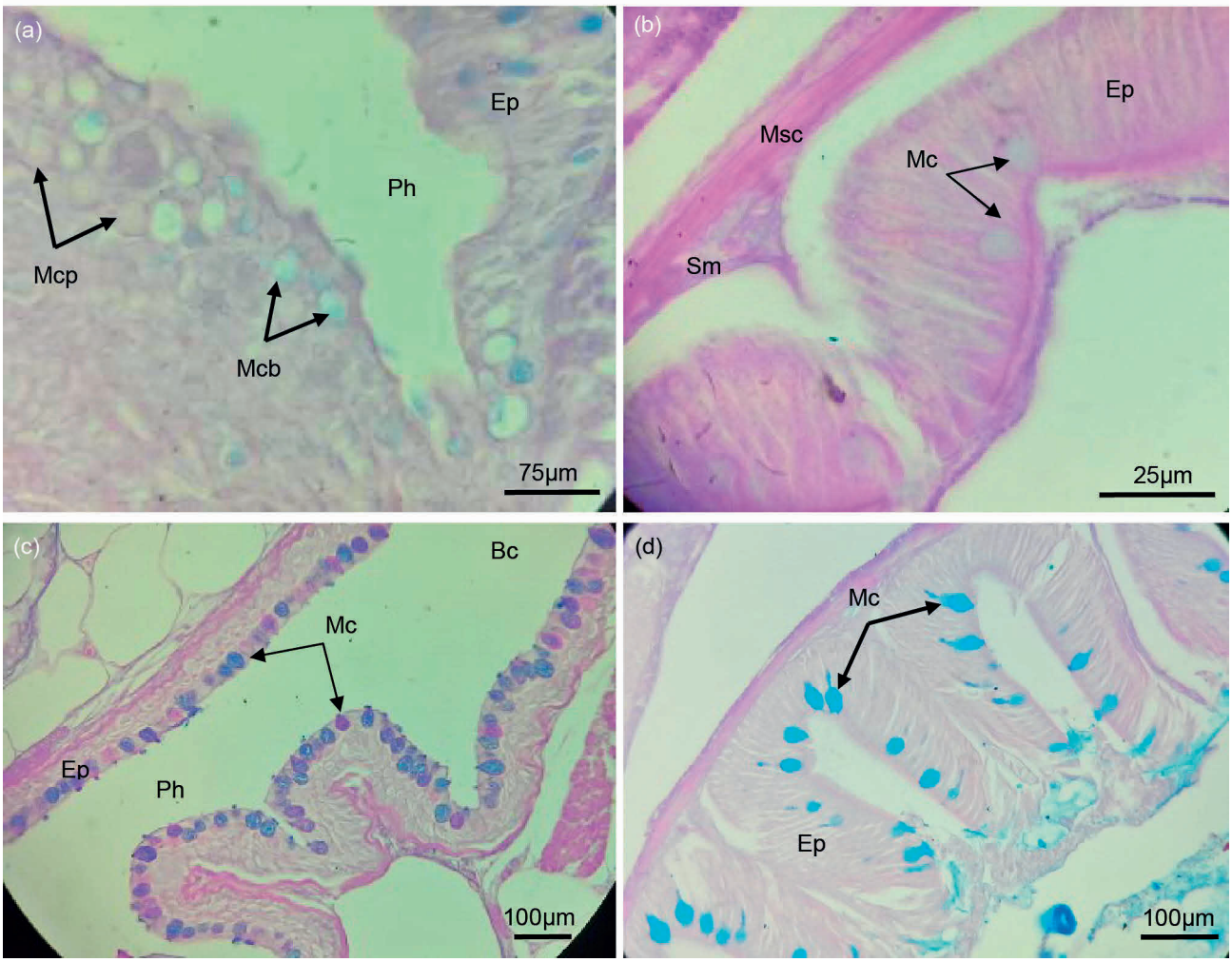

Figure 6: Sections of the digestive system of a *B. altianalis* larva with PAS-AB (pH 2.5) stain showing mucous cells (goblet cells) at various ages. (a) At 7 DAH faint blue coloured mucous cells are observed. (b) Mucous cells at 15 DAH in intestines. (c) Mucous cells are of various stains (intense) at 45 DAH. (d) Intense blue stain mucous cells at 55 DAH. Only blue coloured stains are observed in this region. Sm, submucosa; Mcp, purplish stained mucous cells; Mcb, blue stained mucous cells; Msc, muscularis; Ep, epithelium; Ph, pharynx; Mc, mucous cells; Bc, buccal cavity

8 DAH (W 4.44 ± 0.9 g; TL 1.11 ± 0.02 cm) in fewer larvae. The depletion of the yolk coincided with complete division of the swimbladder by a constriction (Figures 2d and 4), making the animal more capable of active swimming in search of food. On 7 DAH, the mucosal epithelial folds were well patterned and prominent in the proximal region (cranial) of the intestine, but became much more pronounced and grew toward the distal end by 10 DAH (W 5.25 ± 1.25 g; TL 1.16 ± 0.05 cm). The epithelium folds constituted clear pseudocolumnar and columnar cells of variable lengths with large basal nuclei (Figure 5b). The columnar cells were lined by epical eosinophilic brash border (H&E stain). In live larvae observed under the microscope, the entire tract still looked simple and was defined by the presence of *Artemia* eggs (Figures 2c and 2f). The folds were associated with few scattered goblets cells on 6–7 DAH, but increased in number and together with the goblet cells they became numerous by 10 DAH. The intestinal goblets stained very faintly (weakly) blue with PAS and AB (pH 2.5) at 10 DAH. No coloured globules were observed with AB (pH 1.0) along the intestine until 45 DAH when few faintly blue goblet cells were noticed (Figure 6). The brush border along the epithelium was intensely purplish blue with PAS and AB (pH 2.5). At 10 DAH the oesophagus had mucosal epithelial folds with numerous goblets compared with the intestine. By 15 DAH well patterned folds were observed (Figure 2f) and continued to increase in number and length through until 22 DAH (Figure 2g).

Between 28 and 30 DAH the first gut coiling (single bending) was observed. The coiling processes began with a slight bend observed at 22 DAH (Figure 2g) and became pronounced with a clear bend by 30 DAH (Figures 1 and 2h). By 45–55 DAH three sections of the gut were histologically observed in the same slide indicating that the gut had coiled twice (Figure 1). The gut mucosal epithelial folds became more elongated (Table 1) and were well patterned. After each coiling an exponential growth was

observed (Figure 1). The gut coiling coincided with external appearance of glittering colours indicating the beginning of the scale formation process. The scales began forming on the lower part of the lateral line, which was now visible and by 60 DAH the scales were clearly observed above the lateral line and had covered more than 60 percent of the body. At this point the larvae had started metamorphosing into juvenile fish. The coiling of the digestive tract and the presence of scales characterised the fish as a juvenile fish. The mean lengths of epithelial folds, goblet cells and stain tests at various stages of development are provided in Table 1. Table 2 summarises the major developmental processes of the digestive system of the *Barbus altianalis* larvae from 0–60 DAH.

The liver and the pancreas

At hatching the liver and the pancreas were undifferentiated. The liver cells (hepatocytes) were conspicuous 3 DAH as 'pentagonal' or 'hexagonal' transparent cells from live larvae under the light microscope as well as in the histologically processed samples (Figures 2b, 2e, 2h; 3d and 4). They began as very few cells, growing from around the oesophageal region just below the cranial side of the swimbladder, and by 6–7 DAH they had increased in size and number extending toward the posterior region around the gut. By 15 DAH the liver had extended dorsally past the swimbladder and on 45 DAH the liver was very large and had grown up to the posterior end of the gut toward the anus. Histologically, between 0 and 2 DAH the liver and pancreatic tissue could not easily be differentiated, because the cells looked alike. However, on 3 DAH the pancreas was clearly differentiated from the liver by the purplish/reddish zymogen granules that tested positive with PAS-AB stain (Figure 7). From the time the pancreatic cells were observed, they were separated from the liver and by 45 DAH some pancreatic cells were now seen to be embedded within the liver around the blood vessels forming a hepatopancreas as it appears in adult fish (Aruho et al. 2017).

Discussion

Digestion of a compound diet at the start of exogenous feeding by larvae of most cultured species is a bottleneck to mass seed production (Kolkovski 2001; Ostaszewska et al. 2003; Trevino et al. 2011; Hamre et al. 2013). The reason is that the digestive capabilities of larvae of some species at that particular period of exogenous feeding might not be mature or ready to effectively digest and/or absorb required nutrients from microprocessed (compound) diets (Garcia et al. 2001; Zambonino-Ifante et al. 2008). Larval requirements are species specific, age dependent and linked to the development and maturation level of the digestive tract and its associated organs (Kumar et al. 2000; Herrera et al. 2010; Ruan et al. 2013; Zhang et al. 2016).

This study revealed that *B. altianalis* hatched with a big yolk reserve that took 7–8 days to be depleted contrary to the 3 DAH indicated by Rutaisire et al. (2015), hatched at the same temperature of 27 °C. This was confirmed by a histological examination, which was not done in the earlier study. Active feeding started at 5–6 DAH and this implied that there was a period of mixed feeding ranging from 5–8 DAH. In a related cyprinid, *Labeo victorianus*, which was recently domesticated, a mixed feeding period of 2 days from 3 DAH to 5 DAH when yolk was depleted, was observed (Owori 2009). Variations at first feeding by other cultured cyprinids occur (George and Chapman 2013; Zhang et al. 2016) and the amount or size of yolk determined how long the mixed feeding period would last. The longer the larvae take to utilise the yolk reserves, the

Table 2: Summary of the major developmental changes of *Barbus altianalis* larval digestive system from 0 to 60 days after hatching (DAH)

Structure/feature	DAH	Mean total length (cm)
Mouth and anus opening	3–4	0.96 ± 0.05
Period when feeding begins	5–6	1.05 ± 0.05
Yolk sac absorption	7–8	1.1 ± 0.02
Period of mixed feeding	5–8	1.1 ± 0.02
Larvae begin swimming up in search for food	5–6	1.05 ± 0.05
Active swimming starts coinciding with constriction of swimbladder	7–8	1.11 ± 0.02
Complete opening of the gut	5	1.05 ± 0.03
Undifferentiated tissue of liver and pancreas	0–2	0.79 ± 0.032
Clear appearance of liver and pancreas	3	0.95 ± 0.04
Liver grows past the swimbladder	15	1.38 ± 0.089
Liver grows large and extends close to the anus	45	2.34 ± 0.14
Tastebuds, goblets, club cells	2–3	0.91 ± 0.07
Thin intestinal villi	2–3	0.91 ± 0.07
Short pharyngeal folds /increased tastebuds and goblets PAS-AB (pH 2.5) – confirming digestion activity	6–7	0.91 ± 0.07
Goblet cells (mucous cells) increase with intense PAS-AB (pH 2.5) blue/purplish colouration – improved digestion	15	1.38 ± 0.089
Elongated epithelial pharyngeal folds/palatal organ	45	2.34 ± 0.14
Appearance of scales	28–30	1.80 ± 0.11 to 1.95 ± 0.16
Scales cover 60% of the body	60	2.90 ± 0.24
The first intestinal coiling	28–30	1.80 ± 0.11 to 1.95 ± 0.16
The second coiling of intestines	45–55	2.34 ± 0.14 to 2.84 ± 0.26

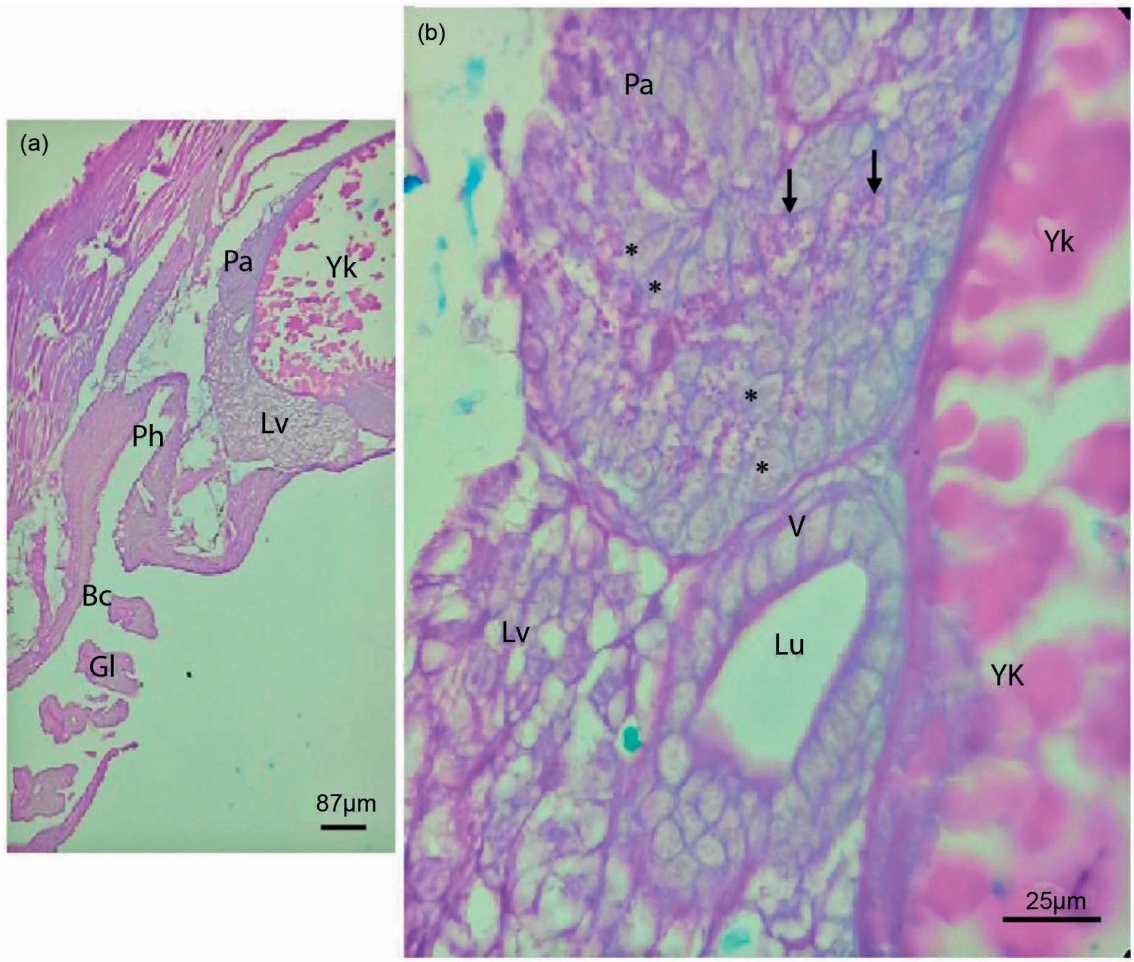

Figure 7: Sections of *B. altianalis* larva showing the differentiation of the liver and the pancreas at 3 DAH. (a) The liver and the pancreas lie over the yolk mass. (b) Magnified section of (a) showing the pancreas with pancreatic acinar (plates) represented by the * (asterisk). The zymogens are shown by the arrows. Bc, buccal cavity; Pa, Pancreas; Lv, liver; Gl, gills; Ph. pharynx; Lu, lumen of the vein; V, vein; Yk, yolk

more it will grow before it searches for its own food sources (Helfman et al. 2009; Rutaisire et al. 2015). This is an advantage in cultured species, because the larvae will grow bigger with a mature digestive system that will quickly and easily accept dry feed at first feeding. The mixed feeding period gives the larvae an advantage to gradually adapt to searching for food when the yolk is completely exhausted (Lucas and Southgate 2012).

The study showed that, despite the fact that the mouth opened 3–4 DAH, no *Artemia* cysts were seen in the gut until 5 DAH implying that feeding immediately in *B. altianalis* after opening of the mouth and the anus could disrupt developmental process of the gut and cause mortalities. This was also reported in Bay snook, *Petenia splendida* (Trevino et al. 2011). The period when exogenous feeding begins is considered the most critical period as delays or early feeding might affect larval growth and survival (Kamler 1992; Mai et al. 2001; Mai et al. 2005). However, this period could be interrupted by temperature, because the rate at which the yolk is depleted will be influenced by temperature. Accelerated temperatures will drain the yolk faster than reduced temperature (Heming 1982; Herrera et al. 2010; George and Chapman 2013). This could make it difficult for aquaculturists to determine the actual period when first feeding begins. In the current study, the beginning of exogenous feeding was visually and morphologically identified when the yolk reduced almost to the level of the head or the lower jaw and this was the same period when the larvae began to lift up from the bottom of the tank with definite constriction of the swimbladder.

Morphological, histological and histochemical examinations revealed that in *B. altianalis*, all the necessary features for digestion were ready by 6–7 DAH. The liver. pancreas, tastebuds, mucous cells, intestinal folds and intestinal goblet cells were present by 6–7 DAH. The presence of neutral and acidic glycoconjugates (mucins) was detected by 7 DAH, implying that they were active in the digestion process. The neutral and sialomucins are characteristic key components of fluid secretions of low viscosity and their presence in the buccopharyngeal cavity largely facilitated lubrication of food items and protection against mechanical abrasion of the mucosal epithelium in fish (Fiertak and Kilarski 2002; Aruho et al. 2017). Sylated glycoconjugates (sialomucins) can

prevent glycosidase degrading activity against the gut epithelium so that the larval gut is protected from infections (Carrasson et al. 2006; Namulawa et al. 2014). However, the highly dense sulphonated mucins were negative with AB (pH 1) stain, hence they were absent in this region at this stage. In mature *B. altianalis,* they are present in the buccopharyngeal cavity and their presence suggests a role in facilitating trapping of small particles and filtration of plankton and other desirable microorganisms or feed particles for feeding in larger juvenile or adult fish (Aruho et al. 2017). Together with the well-developed palatal organ, the sulphomucins are effective in filtering planktons for ingestion by the fish (Sibbing and Uribe 1985). Lack of sulphomucins in the pharyngeal cavity indicates that the filtration process, especially for microparticles, including algae, is poorly developed in larvae, because the palatal organ is immature, and the mechanical filtration process was therefore poor at this stage. This could indicate that the larvae are limited in their mechanical digestive capability but are able to easily ingest relatively larger sized particles than algae. It is presumed that the range of 19–24 µm in the size of *Artemia* or fine larvae powder, as recorded in this study, can be easily captured and ingested, compared with the algae or other smaller particles (<19 µm). The sulphomucins began to appear in the intestine section by 50 DAH, the period when the fish are transforming into juveniles, implying that they had matured and the mode of feeding could have improved. Differences in mucins between age groups (excluding larvae) have also been found in cultured *Cyprinus carpio* and have been attributed to increasing necessity for their role as the fish grows and becomes more diverse in feeding strategies (Neuhaus et al. 2007).

Both neutral and acid mucins were also present along the intestine by 7 DAH, but the acid mucins (sialomucins) were prominently observed staining faintly blue, becoming moderately blue by 14 DAH and intense at 30 DAH, compared with the neutral mucins that stained weakly (or faintly) throughout the experimental period. Neutral mucins are very intense in larvae with mature stomachs where they neutralise the acidity of the HCl acid produced during the digestion of proteins (Namulawa et al. 2014). In stomachless fish, in addition to facilitating quick movement of bolus through the intestine, they are important in preventing the produced enzymes from selfdigestion (Fiertak and Kilarski 2002; Namulawa et al. 2014). It may be postulated that the increasing level of the intensity of mucins as the larvae grew was commensurate with increasing numbers and sizes of tastebuds and goblet cells, lengthening of the intestinal folds, as well as the associated liver and pancreas, together with the enzyme activity resulting in improved digestive capability. Differences in mucin amount in common carp between age groups (excluding larvae) were linked to increased intestinal folds (Neuhaus et al. 2007). The strength of the acid mucins (stain colour strength) was an indicator of improved functionality of mucins, which were vital in facilitating transportation of ions across the epithelium, inducing immunity or defence of the gut and stabilising enzymes (Shephard 1994; Fiertak and Kilarski 2002; Neuhaus et al. 2007; Gomez et al. 2013). The identification of these mucins and the level of gut development at 7 DAH, therefore, suggested that the larvae of *Barbus altianalis* were possibly able to digest a compound diet at first exogenous feeding stage. Despite the fact that some species may directly be weaned to dry (compound) diets at the exogenous feeding, in other species it is possible that more mortalities could still occur even when they can be started on dry feed (Kolkovski 2001; Akbary et al. 2010; Policar et al. 2011; Ramesh et al. 2014). Therefore, this implies that the effectiveness and efficiency of the larval gut system to digest the feed could additionally be tested with various diets, including microdiets to identify and confirm the weaning diet or combination of diets that will provide appropriate nutrients for optimal growth and survival of larvae.

The intestinal coiling followed a relatively sharp rise in larval growth after 28–30 and 45–55 DAH. Variations in the timing of the intestinal coiling in some cultured cyprinids occurred during ontogenetic development of the digestive tract (Ruan et al. 2013). The gut coiling increased the surface area over which digestion and absorption occurred, thereby facilitating rapid growth and development of the larvae (Falk-Petersen 2005; Trevino et al. 2011; Ruan et al. 2013; Rønnestad et al. 2013). The initial gut coiling at 28–30 DAH coincided with initiation of formation of body covering scales and continued even after the experiment was terminated. The process of forming scales is important in protecting the fish from diseases and parasitic infections, facilitating swiftness during swimming, as well as aiding the fish to adapt to various aquatic environments, thereby increasing chances of survival (Creaser 1926; Long et al. 1996; Sherman et al. 2016). This also implied that the larvae had started the transition process to the juvenile stage, a stage when the fish resembled an adult (Jones et al. 1978; Kendall and Moser 1984).

In conclusion, the digestive structure of *B. altianalis* was presumed to be ready to accept the microprocessed diets at first exogenous feeding between 6 and 7 DAH, because all the required gut structures for digestion were present by this period. The digestive structure and its associated organs together with the histochemical characteristic pattern of mucins gradually became prominent as the larvae grew, conferring to the larvae a system for efficient digestion. By 28–30 DAH, the digestive tract had coiled and formed a single loop and at 45–55 DAH it formed a double loop. The intestinal coiling indicated increasing efficiency of the gut system for utilisation of consumed diets and as a result there was a noted increase in the larval growth. Understanding the ontogenetic development process of the larval digestive system is an important step that should be able guide hatchery operators to synchronise the larval nutrition requirements with larvae digestive capability in development of the weaning protocol. Hence, an additional study is required to evaluate the effectiveness of the digestive system with various feeds, including the dry feed from the first exogenous feeding period, aimed at maximising growth and minimising mortalities.

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References

Akbary P, Imanpoor M, Sudagar M, Makhdomi NM. 2010. Comparison between live food and artificial diet on survival rate, growth and body chemical composition of *Oncorhynchus mykiss* larvae. *Iranian Journal of Fisheries Science* 9: 19–32.

Aruho C, Namulawa TV, Tibenda VN, Bugenyi F, Kato CD, Kisekka M, Rutaisire J. 2016. Histo-morphological description of the digestive system of the Rippon Barbel *Barbus altianals* (Boulenger 1900); a potential species for culture. *Uganda Journal of Agricultural Sciences* 17(2): 197–217.

Bancroft JD, Gamble M. 2002. *Theory and practice of histological techniques* (5th edn. pp 85–107). Edinburgh, London, United Kingdom: Churchill Livingston Publishers.

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

Carrasson M, Grau A, Dopazo LR, Crespo S. 2006. A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). *Histology and Histopathology* 21: 579–593.

Chemoiwa EJ, Abila R, Macdonald A, Lamb J, Njenga E, Barasa JE. 2013. Genetic diversity and population structure of the endangered Ripon barbel, *Barbus altianalis* (Boulenger, 1900) in Lake Victoria catchment, Kenya based on mitochondrial DNA sequences. *Journal of Applied Ichthyology* 29: 1225–1233.

Chen BN, Qin JG, Kumar MS, Hutchinson W, Clarke S. 2006. Ontogenetic development of the digestive system in yellowtail kingfish *Seriola lalandi* larvae. *Aquaculture (Amsterdam, Netherlands)* 256: 489–501.

Creaser CW. 1926. The structure and growth of the scales of fishes in relation to the interpretation of their life-history, with special reference to the sunfish *Eupomotis gibbosus* (No. 17). Michigan, USA: Museum of Zoology, University of Michigan, Miscellaneous publication 17: 1–82.

De Silva SD, Anderson TA, Sargent JR. 1995. Fish nutrition in aquaculture. *Reviews in Fish Biology and Fisheries* 5: 472–473.

Falk-Petersen IB. 2005. Comparative organ differentiation during early life stages of marine fish. *Fish and Shellfish Immunology* 19: 397–412.

Fiertak A, Kilarski WM. 2002. Glycoconjugates of the intestinal goblet cells of four cyprinids. *Cellular and Molecular Life Sciences* 59: 1724–1733.

Garcia-Hernandez M, Lozano MT, Elbal MT, Agulleiro B. 2001. Development of the digestive tract of sea bass (*Dicentrarchus labrax* L). Light and electron microscopic studies. *Anatomy and Embryology* 204: 39–57.

George AE, Chapman DC. 2013. Aspects of embryonic and larval development in bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *PLoS One* 8(8): e73829.

Gomez D, Sunyer, JO, Salinas I. 2013. The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. *Fish & Shellfish Immunology* 35: 1729–1739.

Hamlin HJ, Herbing IH, Kling LJ. 2000. Histological and morphological evaluations of the digestive tract and associated organs of haddock throughout post-hatching ontogeny. *Journal of Fish Biology* 57: 716–732.

Hamre K, Yufera M, Rønnestad I, Boglione C, Conceiçao LE, Izquierdo M. 2013. Fish larval nutrition and feed formulation: Knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in Aquaculture* 5: S26–S58.

Helfman G, Collette BB, Facey DE, Bowen BW. 2009. *The diversity of fishes: biology, evolution, ecology*. Chichester, UK: John Wiley and Sons.

Heming TA. 1982. Effects of temperature on utilisation of yolk by Chinook salmon (*Oncorhynchus tshawytscha*) eggs and alevins. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 184–190.

Herrera M, Hachero-Cruzado I, Naranjo A, Mancera JM. 2010. Organogenesis and histological development of the wedge sole *Dicologoglossa cuneata* M. larva with special reference to the digestive system. *Reviews in Fish Biology and Fisheries* 20: 489–497.

Hopkins KD. 1992. Reporting fish growth: a review of the basics. *Journal of the World Aquaculture Society* 23: 173–179.

Jones PW, Martin FD, Hardy JD, 1978. Development of fishes of the mid-Atlantic bight: an atlas of egg, larvae, and juvenile stages, Acipenseridae through Ictaluridae. Report No. FWS/OBS-78/12, Volume 1. (366 p). Ft. Collins, CO, USA: US Department of the Interior, Fish and Wildlife Service, Office of Biological Services Program.

Kamler E. 1992. Endogenous feeding period. Early life history of fish. In: Chapman and Hall (Eds). *An energetic approach. Fish and fisheries series* (IV: 107–196). London, United Kingdom: Chapman and Hall.

Kendall AW, Ahlstrom EH, Moser HG. 1984. Early life history stages of fishes and their characters. In: Moser HG, Richard WJ, Cohen DM, Fahay MP, Kendall AW, Richardson SL (Eds). American Society of Ichthyologist and Herpetologists, special publication 1. (p 11–22). Lawrence, Kansas, USA: American Society of Ichthyologist and Herpetologists.

Kolkovski S. 2001. Digestive enzymes in fish larvae and juveniles-implications and applications to formulated diets. *Aquaculture (Amsterdam, Netherlands)* 2000: 181–201.

Kumar S, Sharma JG, Chakrabarti R. 2000. Quantitative estimation of proteolytic enzyme and ultrastructural study of anterior part of intestine of Indian major carp (*Catla catla*) larvae during ontogenesis. *Current Science-Bangalore* 79: 1007–1010.

Long J, Hale M, Mchenry M, Westneat M. 1996. Functions of fish skin: flexural stiffness and steady swimming of longnose gar, *Lepisosteus osseus*. *The Journal of Experimental Biology* 199: 2139–2151.

Lucas JS, Southgate PC (Eds). 2012. *Aquaculture: Farming aquatic animals and plants* (pp 629). West Sussex, UK: Wiley-Blackwell.

Mai K, Yu H, Ma H, Duan Q, Gisbert E, Zambonino-Infante JL, Cahu CL. 2005. A histological study on the development of the digestive system of *Pseudosciaena crocea* larvae and juveniles. *Journal of Fish Biology* 67: 1094–1106.

Namulawa VT, Kato CD, Nyatia E, Kiseka M, Rutaisire J. 2014. Histochemistry and PH Characterization of the Gastrointestinal Tract of Nile Perch *Lates niloticus*. *World Journal of Fish and Marine Sciences* 6: 162–168.

Neuhaus H, Van der Marel M, Caspari N, Meyer W, Enss M L, Steinhagen D. 2007. Biochemical and histochemical study on the intestinal mucosa of the common carp *Cyprinus carpio* L, with special consideration of mucin glycoproteins. *Journal of Fish Biology* 70: 1523–1534.

Ostaszewska T, Wegner A, Wegiel M. 2003. Development of the digestive tract of Ide, *Leuciscus idus* (L) during the larvae stage. *Archives of Polish Fisheries* 11: 79–92.

Owori WA. 2009. The feeding ecology, ontogeny and larval feeding in *Labao Victorianus* Boulenger 1901 (Pisces: Cyprinidae). PhD thesis. Makerere University, Uganda.

Pearse AGE. 1985. *Histochemistry-Theoretical and applied*. Vol. II. Edinburgh (pp 441–1055). London, United Kingdom. Churchill Livingstone Inc.

Policar T, Podhorec P, Stejskal V, Kozak P, Svinger V, Hadi Alavi SM. 2011. Growth and survival rates. puberty and fecundity in captive common barbel (*Barbus barbus* L.) under controlled condition. *Czech Journal of Animal Science* 56: 433–442.

Ramesh R, Dube K, Prakash C, Tiwari VK, Rangacharyulu PV, Venkateshwarlu G. 2014. Growth and survival of pengba, *Osteobrama belangeri* (Val.) larvae in response to co-feeding with live feed and microparticulate diet. *Ecology Environment and Conservation* 20: 1715–1721.

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

Rønnestad I, Yufera M, Ueberschar B, Ribeiro L, Sæle O, Boglione C. 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, gaps and bottlenecks in research. *Reviews in Aquaculture* 5: S59–*S*98.

Ruan GL, Yang DQ, Wang WM. 2013. Ontogeny of the digestive tracts in grass carp (*Ctenopharyngodon idella*), yellowcheck carp (*Elopichthys bambusa*) and topmouth culter (*Culter alburnus*). *Shui Sheng Sheng Wu Hsueh Bao* 36: 1164–1170.

Rutaisire J, Levavi-Sivan B, Aruho C, Ondhoro CC. 2015. Gonadal recrudescence and induced spawning in *Barbus altianalis*. *Aquaculture Research* 46: 669–678.

Sarasquete MC, Polo A, Yufera M. 1995. Histology and histochemistry of the development of the digestive system of larval gilthead seabream, *Sparus aurata* L. *Aquaculture (Amsterdam, Netherlands)* 130: 79–92.

Shephard KL. 1994. Functions for fish mucous. *Reviews in Fish Biology and Fisheries* 4: 401–429.

Sherman VR, Yaraghi NA, Kisailus D, Meyers MA. 2016. Microstructural and geometric influences in the protective scales of *Atractosteus spatula*. *Journal of the Royal Society Interface* 13. https://doi.org/10.1098/rsif.2016.0595.

Sibbing FA, Uribe R. 1985. Regional specialisation in the oral-pharyngeal wall and food processing in carp (*Cyprinus carpio* L.). *Netherlands Journal of Zoology* 35: 377–422

Snoeks J, Kaningini B, Masilya P, Nyina-Wamwiza L, Guillard J. 2012. Fishes in Lake Kivu: diversity and fisheries. pp 127–152. In: Descy JP, Darchambeau F, Schmid M, (Eds), *Limnology and biogeochemistry of a tropical great lake, Aquatic Ecology Series.* Dordrecht, Netherlands: Springers

Trevino L, Alvarez-Gonzalez CA, Perales-García N, Arevalo-Galan L, Uscanga-Martínez A, Marquez-Couturier G, Fernandez I, Gisbert E. 2011. A histological study of the organogenesis of the digestive system in bay snook *Petenia splendida* Gunther, 1862 from hatching to the juvenile stage. *Journal of Applied Ichthyology* 27: 73–82.

Zambonino-Infante J, Gisbert E, Sarasquete C, Navarro I, Gutierrez J, Cahu CL. 2008. Ontogeny and physiology of the digestive system of marine fish larvae. pp 281–344. In: Cyrino JEO, Bureau D, Kapoor BG (Eds), *Feeding and Digestive Functions of* Fish. Enfield, USA: Science Publishers Inc.

Zhang J, Yang R, Yang X, Fan Q, Wei K, Wang W. 2016. Ontogeny of the digestive tract in mud loach *Misgurnus anguillicaudatus* larvae. *Aquaculture Research* 47: 1180–1190.

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