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Organogenesis of the digestive system in Neotropical carnivorous freshwater catfish *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae)☆

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ABSTRACT

The morphological development of the digestive system of *Hemisorubim platyrhynchos* was studied from the day of hatching until 21 days post-hatching (DPH) using histology, histochemistry and scanning electron microscopy to augment the available knowledge regarding the organogenesis of the digestive system of this carnivorous Neotropical fish. The development of the digestive system was divided into four major stages. Stage I (endotrophic period) starts with hatching and ends with the mouth opening at 2 DPH. The digestive tract originated as a straight undifferentiated tube and ended as an esophagus with goblet cells, an incipient stomach and an intestine divided into the anterior, middle, posterior and rectum. Stage II (endo–exotrophic period) is from the onset of feeding to exhaustion of the yolk at 4 DPH. Stage III is the period in which the larvae rely exclusively on exogenous feeding but still have no functional stomach. Stage IV is an exotrophic period marked by the appearance of gastric glands at 15 DPH. At 20 DPH, the saccular stomach can be observed. Fish growth was largely variable over the time period studied, and the variability was predominant between the period in which the yolk was present and after its exhaustion. The mixed feeding period is short, and the larvae subsequently survive solely by exogenous feeding without a functional stomach for 15 days. During this period, the primary site of digestion is the anterior intestine, which presents with a saccular shape. The accessory glands, liver and pancreas were differentiated at 2 DPH and thus contributed to extracellular digestion. Also observed in the intestine were supranuclear inclusions that could promote intracellular digestion. The rectal columnar epithelium showed scarce goblet cells but had apical mucosubstances that were involved in fecal transit, epithelial protection and in the final absorption of substances. Gastric glands appeared at 15 DPH and, until this period, the larvae should receive live feed and after 15 DPH may be weaned. *H. platyrhynchos* farmers should also be alert to differences in the size of the fish because this species presents cannibalism and needs to be separated into homogeneous batches.

1. Introduction

Ontogenetic studies during the early stages of fish development, primarily organogenesis of the digestive system, are valuable tools for better understanding of larval nutritional physiology and establishing rearing protocols. According to Rønnestad et al. (2013), integrated understanding of the various factors and events that interact during food acquisition and digestion is necessary for designing diets that meet the requirements for optimal ingestion, digestion and absorption. In this case, the health and quality of spawners and environmental conditions during egg incubation determine the amount and quality of endogenous reserves (yolk) of larvae, and environmental and feeding conditions affect the rate of larval development (Hachero-Cruzado et al., 2009). Fish larvae need their feeding and digestive systems to develop quickly. Once the endogenous reserves are depleted, they need to begin capturing, ingesting and digesting food to provide fuel for the intense processes of metabolism and growth (Cuenca-Soria et al., 2013). The basic mechanisms of organ and system development

☆ Statement of relevance. This study contributes to the knowledge of the digestive tract to improve carnivorous freshwater fish rearing. Sensory structures of *Hemisorubim platyrhynchos* larvae appeared at 2 DPH. *H. platyrhynchos* has an esophagus with goblet cells and a saccular intestine at the onset of exogenous feeding. Larvae depend exclusively on exogenous feeding at 5 DPH, but gastric glands appeared at 15 DPH and, then, larvae could be weaned.

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are similar in all teleosts, even though there are considerable interspecies differences regarding the relative timing of differentiation, development and functionality during early ontogeny (Treviño et al., 2011). According to Rønnestad et al. (2013), the variety in ontogeny, feeding physiology and behavior even within the same family should be taken into account. Thus, species-specific findings for a process or function in a model species cannot be extrapolated directly to other teleosts, and specific validation studies are essential. Several studies have analyzed the development of the digestive system in fish, including in *Channos chanos* (Ferraris et al., 1987), *Sparus aurata* (Sarasquete et al., 1995), *Pleuronectes ferruginea* (Baglole et al., 1997), *Mugil platunus* (Galvão et al., 1997), *Paralichthys californicus* (Gisbert et al., 2004), *Pangasius sutchi* (Islam, 2005), *Seriola lalandi* (Chen et al., 2006), *Clarias gariepinus* (Osman et al., 2008), *Rhamdia quelen* (Amorim et al., 2009), *Scophthalmus rhombus* (Hachero-Cruzado et al., 2009), *Ompok bimaculatus* (Pradhan et al., 2012), *Trachinotus ovatus* (Ma et al., 2014) and *Thunnus thynnus* (Yúfera et al., 2014). Although there are many larvae studies, knowledge of the ontogeny of the digestive system of Neotropical fish is limited to a few species (Portella et al., 2014). Thus, understanding the functions and limitations in processing capacity of the digestive system during the early stages of life continues to be a key to the success of larviculture, with the timing of organ development and how it relates to digestion critical to the generation of better rearing systems (Rønnestad et al., 2013).

Hemisorubim platyrhynchos belongs to the family Pimelodidae and the order Siluriformes. This is a migratory species without parental care that is widely distributed in the Neotropical region, with reports indicating its presence in the Orinoco, Amazon, Paraguay, Uruguay and Paraná River basins. According to Bressan et al. (2009), *H. platyrhynchos* is a nocturnal carnivorous fish, and the population size of this species has been reduced because of habitat destruction primarily as a consequence of the construction of hydroelectric dams that interrupt the flow of migration required for reproduction. This species is valuable for aquaculture because of the quality and flavor of its meat and its absence of intramuscular bones. Thus, the aim of this study was to describe the organogenesis of the digestive system of *H. platyrhynchos* from hatching to 21 days of life for a better understanding of morphofunctional aspects of digestion in Neotropical carnivorous fish larvae. This study will be useful in guiding feed management protocols and farming techniques to increase the production and efficiency of larviculture.

2. Materials and methods

2.1. Rearing conditions, sampling and growth measurements

H. platyrhynchos gametes were obtained by induced spawning in the Hydrobiology and Aquaculture Station of São Paulo Energetic Company (CESP), Jupiá, SP, Brazil. Fertilized eggs (85% fertilization rate) were incubated at 29.2 ± 1.0 °C, and hatching occurred at 15–17 h post-fertilization (95% hatching rate). During the period studied, the levels of dissolved oxygen (7.3 ± 0.5 mg/l), pH (8.2 ± 0.5) and ammonia (12.5 ± 1.0 μg/l) were controlled. Larvae were fed *Artemia* sp. since 3 DPH. Specimens were collected ($n = 15$) at 4-h intervals from hatching to 5 days post-hatching (DPH) and at one-day intervals thereafter until 21 DPH. For sampling, the specimens were euthanized (overdose of benzocaine) and fixed for 24 h at 4 °C in a solution of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4). After fixation, the samples were analyzed, measured for the standard length (SL) (i.e., from the jaw to the end of the notochord) and documented using an M50 stereomicroscope (Leica, Germany). The animals were measured and the growth was determined by the absolute growth rate (AGR) as mm/day and specific growth rate (SGR) as %/day (Hopkins, 1992). AGR was calculated by $AGR = (SL_f - SL_i)/\Delta t$, and SGR was determined by $SGR = 100^*(LnSL_f - LnSL_i)/\Delta t$, where SL_f and SL_i are the final and initial standard length (mm) of the fish, respectively, and Δt is the time interval (days) between sampling (Hopkins, 1992).

2.2. Histological and histochemical analysis procedures

Specimens were dehydrated in graded ethanol solutions and embedded in paraplast (Oxford, USA) or methacrylate resin (Leica, Germany). Sagittal or transverse sections of paraplast (3–7 μm thick) or methacrylate resin (2–3 μm thick) were submitted for staining with hematoxylin-eosin (HE) and 1% toluidine blue (TB). For histochemical tests, periodic acid-Schiff (PAS) was used to stain neutral mucins, and alcian blue (AB) pH 1.0 and 2.5 was used to stain acidic mucins (Suvarna et al., 2013). Sections were analyzed and photo-documented using a BX50 microscope (Olympus, Japan).

2.3. Ultrastructural analysis procedures using a scanning electron microscope

After fixation, the samples were post-fixed for 2 h in 1% osmium tetroxide (pH 7.4) and dehydrated in a graded ethanol solutions. These fragments were processed to obtain the critical point (EMS 850, Electron Microscopy Sciences, USA) and sputtered with colloidal gold (Vacuum Desk II, Denton Vacuum, USA). Analysis and photographic documentation were performed using a Quanta 200 scanning electron microscope (Fei, USA) at the Electron Microscopy Center of the Institute of Biosciences of Botucatu, UNESP, Brazil.

2.4. Ethical note

This study was approved by the Ethical Committee for Research of the Faculty of Sciences at São Paulo State University–UNESP, Bauru, SP, Brazil, under protocol no. 565/46/01/10.

3. Results

3.1. Growth and initial development

At hatching, *H. platyrhynchos* presented a mean standard length (SL) of 3.23 ± 0.10 mm and increased to 7.90 ± 0.68 mm at 21 DPH (Fig. 1). The absolute growth rate (AGR) during the period studied was 0.22 mm/day, and the specific growth rate (SGR) was 4.26%/day. However, from hatching to 5 DPH (with yolk), the AGR was 0.60 mm/day, and the SGR was 13.80%/day. From 5 to 21 DPH (yolk exhausted), the AGR was 0.13, and the SGR was 2.01%/day. The ontogenetic development of *H. platyrhynchos* was divided into four major stages. Stage I (endotrophic period) starts with hatching (SL 3.23 ± 0.10 mm) and ends with mouth opening at 2 DPH (Fig. 1A and B). At hatching, the digestive tract is a straight tube; at the end of stage I, the esophagus with goblet cells, the incipient stomach and the intestine, which were divided into anterior, middle, posterior and rectum sections, were observed. Stage II is the endo–exotrophic period from the onset of feeding (3 DPH, SL 4.88 ± 0.13 mm) to exhaustion of the yolk (4 DPH) (Fig. 1C and D). Stage III (5 DPH, SL 5.61 ± 0.10 mm, to 14 DPH) is the period in which the larvae rely exclusively on exogenous feeding but still have no functional stomach (Fig. 1E and F). In this stage, the intestine increased in length and the middle intestine began to loop. Stage IV is an exotrophic period marked by the appearance of gastric glands at 15 DPH (SL 7.44 ± 0.70 mm) (Fig. 1G). At 20 DPH, the saccular stomach can be observed (Fig. 1H and I).

Upon hatching, the larvae were transparent with a yellowish yolk and chromatophores in the anterior and posterior regions of the yolk sac (Fig. 1A). The digestive tract was a straight tube located dorsally to the yolk sac with the end portion bent ventrally (Fig. 1B). A few hours after hatching, the barbels, eyes and nostrils were observed (Fig. 1C and D). During the growth of the fish, the digestive tract increased in length, primarily in the intestine, which expanded the lumen and

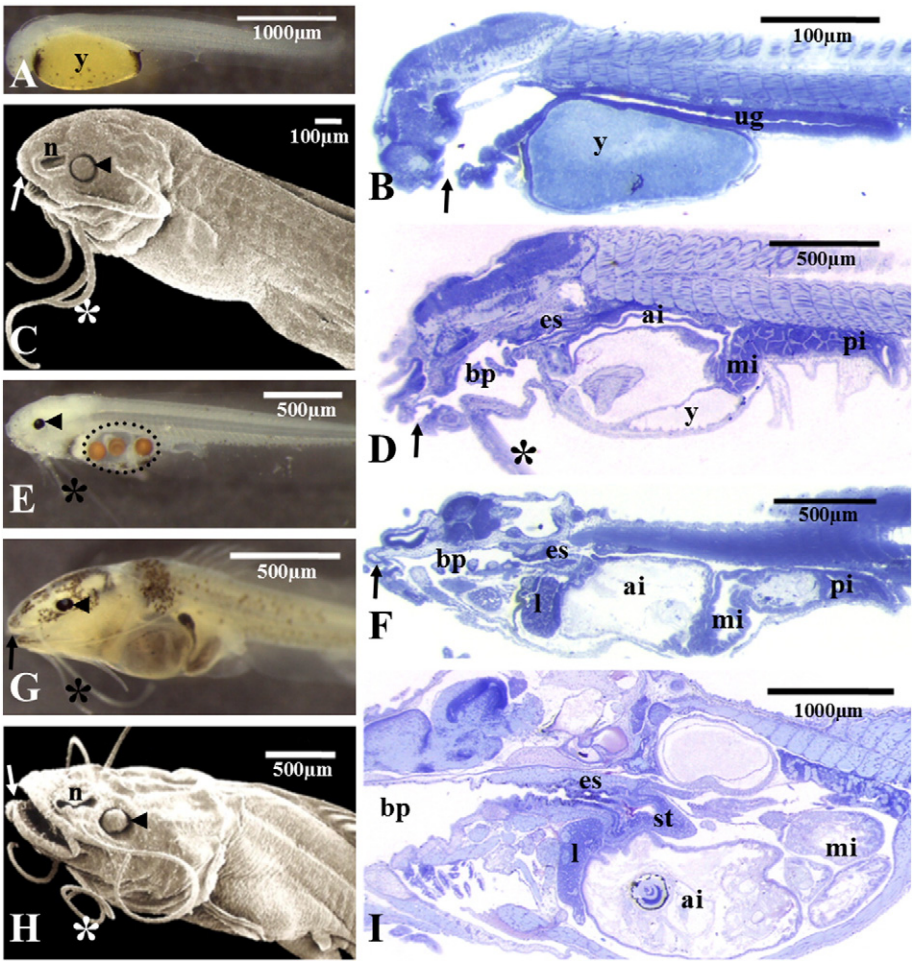

Fig. 1. Morphological development of *H. platyrhynchos*, from hatching to 21 days post-hatching (DPH). (A) Stereomicrograph of *H. platyrhynchos* at hatching (stage I), evidencing the yellow yolk sac (y). (B) Histological section at hatching, note the undifferentiated gut (TB). (C) Electron micrograph at 2 DPH, showing the barbells, mouth, eyes and nostrils. (D) Larvae at 3 DPH (stage II), evidencing the yolk and the digestive tract divided into the buccopharynx, esophagus and anterior, middle and posterior intestine (TB). (E) Larvae at 6 DPH (stage III), evidencing food in the intestine. (F) Histological section of larvae at 5 DPH (stage III), note the saccular anterior intestine (TB). (G) Stereomicrograph of the larvae at 15 DPH (stage IV). (H) Electron micrograph of the larvae at 21 DPH (stage IV). (I) Histological section at 21 DPH, showing the saccular stomach (TB). Abbreviations: ai, anterior intestine; bp, buccopharynx; es, esophagus; l, liver; mi, middle intestine; n, nostrils; pi, posterior intestine; st, stomach; ug, undifferentiated gut; y, yolk; arrow, mouth; arrowhead, eyes; asterisk, barbels.

showed a saccular shape (Fig. 1F). At this moment, the larvae already had their mouths open, and they had developed their sensory structures (i.e., barbels, eyes and nostrils). The swim bladder starts to inflate at 2 DPH. At the end of the experimental period, the digestive tract consisted of the buccopharynx, esophagus, stomach (cardiac, fundic and pyloric) and intestine (anterior, middle, posterior and rectum) (Fig. 1H). The pyloric caeca were absent in this specie. During the studied period, *H. platyrhynchos* also presented with cannibalistic behavior, as two small larvae were found in the anterior intestine of a larger larva. The main ontogenetic events observed in this study are summarized in Table 1.

3.2. Buccopharynx

At hatching, the buccopharyngeal cavity was present and lined by a simple squamous epithelium surrounded by a thin layer of connective tissue (Fig. 1B). At 2 DPH, the mouth opened in a ventral position, and the first teeth were observed, increasing in number with growth of the fish (Fig. 1D). The mouth arrived at the terminal position at 4 DPH. The first goblet cells appeared before mouth opening, and they remained scattered even with fish growth (Fig. 2A). Goblet cells showed reactivity with both PAS and AB (Fig. 2B and Table 2). Taste buds were also observed in the buccopharyngeal cavity at 3 DPH (Fig. 2A). Using histochemical techniques, the taste buds presented with the strongest

Table 1
Main morphological events in development of *H. platyrhynchos*, from the hatching to 21 DPH.

Stage	DPH	Food source	SL (mean ± SD)	Main morphological events
I	0–2	Endogenous	3.23 ± 0.10	Goblet cells in buccopharynx and esophagus Mouth opening First teeth Anterior intestine saccular Liver and pancreas developed Barbell developed
II	3–4	Endo–exogenous	4.88 ± 0.13	Intestine: anterior, middle, posterior and rectum Goblet cells in intestine Longitudinal folds in esophagus First taste buds Mouth in terminal position Yolk exhausted
III	5–14	Exogenous	5.61 ± 0.10	Loops in the middle intestine
IV	15–21	Exogenous	7.44 ± 0.70	First gastric glands Saccular stomach Gastric epithelial cells with neutral mucins

Days post-hatching (DPH); mean standard length (SL) in first day of stage; standard deviation (SD).

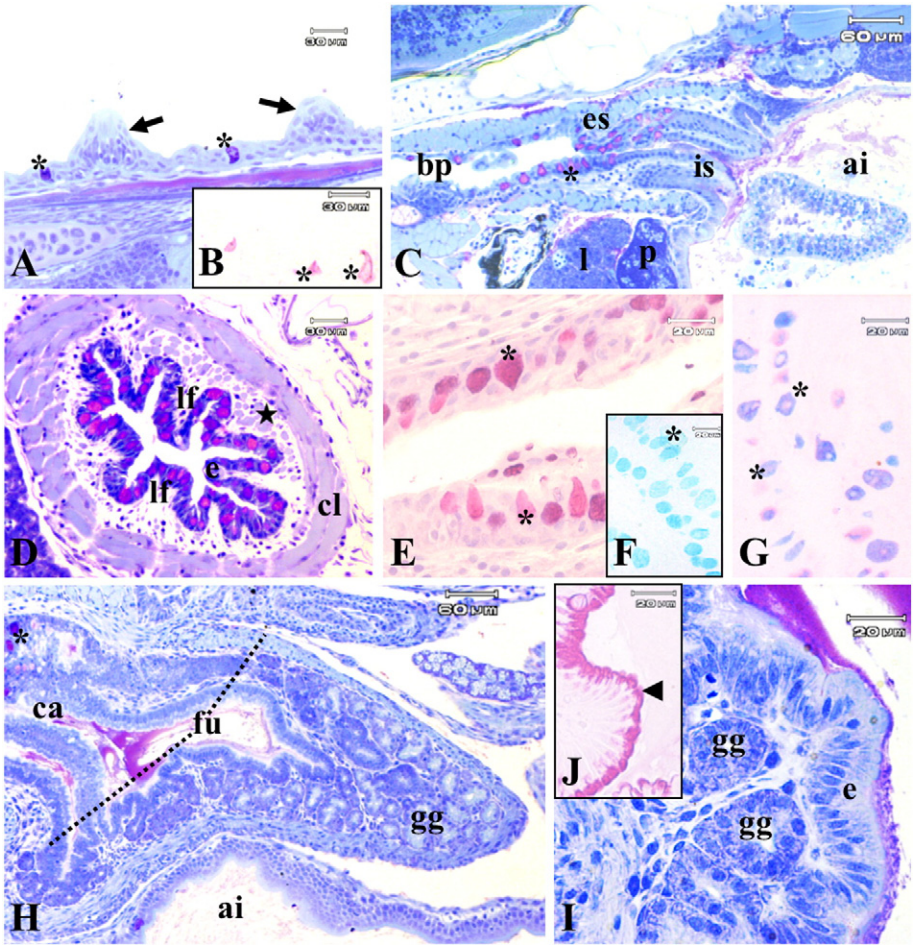

Fig. 2. Histological and histochemical section of the buccopharynx, esophagus and stomach of *H. platyrhynchos* larvae. (A) Longitudinal section of the buccopharynx of *H. platyrhynchos* larvae at 4 days post-hatching (DPH), showing taste buds and goblet cells (TB). (B) Scattered goblet cells PAS-positive in the buccopharynx of larva with 10 DPH. (C) Larva with 3 DPH showing esophagus with numerous goblet cells, the incipient stomach and anterior intestine (TB). (D) Transversal section of the esophagus larva with 5 DPH, evidencing longitudinal folds, the stratified squamous epithelium with numerous goblet cells and the tunica muscularis with an inner longitudinal and outer circular layer (TB). (E, F and G) Histochemical analysis of the goblet cells of esophagus: note the strong reaction to PAS (E), AB pH 2.5 (F) and association between neutral and acidic mucosubstances (G). (H) Larva at 21 DPH, evidencing the saccular stomach with numerous gastric glands in the cardiac and fundic regions (TB). (I) Gastric mucosa of larva at 21 DPH; note the columnar epithelium and tubular gastric glands (TB). (J) PAS reaction in the apical portion of gastric epithelial cells. Abbreviations: ai, anterior intestine; bp, buccopharynx; ca, cardiac region; cl, circular layer of muscularis; e, epithelium; es, esophagus; fu, fundic region; gg, gastric glands; is, incipient stomach; l, liver; lf, longitudinal folds; p, pancreas; arrow, taste bud; arrowhead, apical mucins of gastric epithelial cells; asterisk, goblet cells; star, longitudinal layer of muscularis.

reaction to PAS and AB. As the fish grew, the buccopharyngeal cavity epithelium became a stratified squamous epithelium (Fig. 2A).

3.3. Esophagus

Before the onset of exogenous feeding, a short and rudimentary esophagus was observed to be lined by a stratified squamous epithelium with scattered goblet cells. As the fish grew, the esophageal mucosa showed longitudinal folds, and the goblet cells increased in number (Fig. 2C and D). Histochemical analysis revealed that goblet cells were PAS- and AB-positive starting at 2 DPH (Fig. 2E–G; Table 2). The wall of the esophagus became thick, mainly by the tunica muscularis, which was composed of an inner longitudinal and an outer circular striated muscular layer (Fig. 2C and D). The end of the esophagus was marked by an abrupt change in the stratified squamous epithelium with numerous goblet cells to a simple columnar gastric epithelium without goblet cells (Fig. 2C). Taste buds were not observed in the esophagus.

Table 2
Histochemical analysis in the digestive system of *H. platyrhynchos* larvae at 21 DPH (stage IV).

	Regions							
Techniques employed	Esophagus	Stomach (epithelium)	Stomach (glands)	Anterior intestine	Middle intestine	Posterior intestine	Rectum	Liver
PAS	+++	+++	+	+++	+++	+++	+++	+++
AB pH 2.5	+++	+	−	++	++	+++	++	−
AB pH 1.0	+++	−	−	+	++	+++	++	−
AB (pH 2.5) + PAS	++	−	−	+	++	++	++	−
Cellular types	Goblet cells	Epithelial cells	Oxynticopeptic cells	Goblet cells	Goblet cells	Goblet cells	Epithelial cells	Hepatocytes

Staining intensity: (−) negative; (+) weak; (++) moderate; (+++) strong.

3.4. Stomach

The stomach was the last organ in the digestive system to begin differentiation. At 2 DPH, the incipient stomach was observed between the esophagus and the saccular anterior intestine (Fig. 2C). The first gastric glands appeared at 15 DPH, but the saccular J-shaped stomach was only observed at 20 DPH. At this time, the stomach was divided into three regions: the cardiac, fundic and pyloric (Fig. 2H). The gastric mucosa was lined by simple columnar epithelium with nuclei in the basal third of the cells (Fig. 2H and I). The gastric epithelial cells demonstrated apical neutral mucins revealed by the PAS technique (Fig. 2J; Table 2). The gastric glands were numerous in the fundic region, were scarce in the cardiac region and were not observed in the pyloric region. These glands are tubular and surrounded by connective tissue (Fig. 2H). The submucosa was thin and constituted of dense connective tissue. The tunica muscularis was subdivided into an inner circular and an outer longitudinal layer. In the pyloric region, the circular layer became thicker to form the pyloric sphincter.

3.5. Intestine

At hatching, the straight intestine was lined by a simple columnar epithelium, and the end portion was bent ventrally. As the fish grew, the intestine presented with many folds. At 3 DPH, it was constituted by four regions: anterior, middle, posterior and rectal (Fig. 1F). The anterior intestine expanded the lumen, presenting with a saccular shape. The middle intestine possessed loops and a dilated lumen. The posterior region was straight with many longitudinal folds, whereas the rectal region did not have any folds (Fig. 3D). The intestine showed a simple columnar epithelium, but in the anterior intestine, the columnar cells were shorter than in other regions (Fig. 3A–C). Enterocytes showed a basal nucleus and an apical brush border that was PAS positive. At 4 DPH, supranuclear inclusions were also observed in the enterocytes, mainly in the middle and posterior intestine (Fig. 3B). At this time, few goblet cells were present between enterocytes, showing PAS and AB-positive secretory granules (Fig. 3F and G; Table 2). As the fish grew, these cells increased in number and were numerous in the posterior intestine but scarce or absent in the rectum (Fig. 3D). In the rectal epithelium, the epithelial cells showed an apical secretion (Fig. 3E) that was reactive by both PAS and AB (Table 2).

3.6. Liver and pancreas

The accessory glands of the digestive system were undifferentiated at hatching, being observed only as a cluster of spherical cells in the ventral region of the digestive tract cranial to the yolk sac. These cells

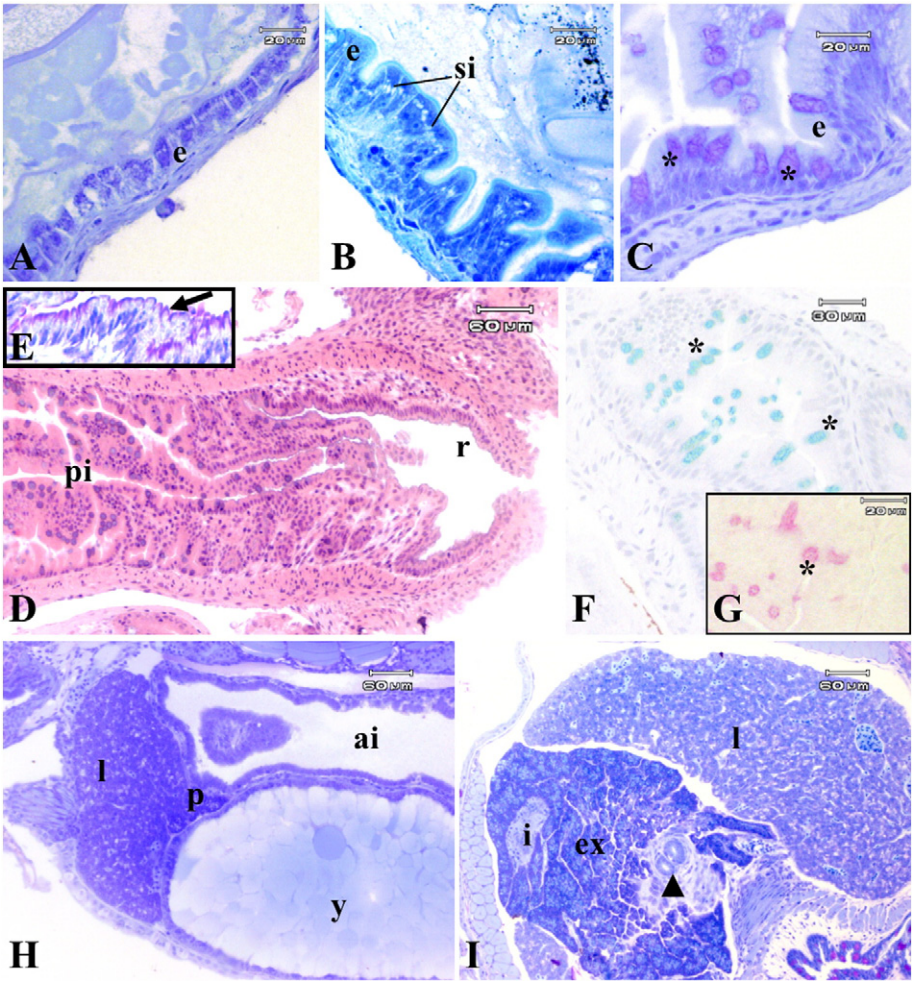

Fig. 3. Histological and histochemical section of the intestine, liver and pancreas of *H. platyrhynchos* larvae. (A) Anterior intestine at 15 days post-hatching (DPH); note the low columnar epithelium (TB). (B) Middle intestine; note the high columnar epithelium (TB). (C) Posterior intestine epithelium; note the high columnar epithelium with numerous goblet cells and the supranuclear inclusion in the enterocytes (TB). (D) Posterior intestine with longitudinal folds and rectum without folds neither goblet cells (HE). (E) Rectal epithelium, evidencing the apical mucins in the epithelial cells (TB). (F and G) Histochemical analysis of goblet cells in posterior intestine; note the strong reaction to AB pH 2.5 (F) and PAS (G). (H) Liver and pancreas formed at 2 DPH; note the large amount of yolk (TB). (I) Transversal section of the accessory glands of digestive system of *H. platyrhynchos*, evidencing the liver, bile duct and exocrine pancreas with acinar cells and endocrine pancreas composed by islet organ (TB). Abbreviations: ai, anterior intestine; e, epithelium; ex, exocrine pancreas; i, islet organ; l, liver; p, pancreas; pi, posterior intestine; r, rectum; si, supranuclear inclusion; y, yolk; arrow, apical mucins of epithelial in rectum; asterisk, goblet cell; arrowhead, bile duct.

increased rapidly in number, and then the liver and pancreas were visualized at 1 DPH (Fig. 3H). Hepatocytes were polyhedral cells with rounded nuclei (Fig. 3I), and the cytoplasm presented with PAS-positive glycogen granules (Table 2). The extrahepatic pancreas was observed on the visceral face of the liver (Fig. 3H and I). This pancreatic tissue was comprised of acinar cells of the exocrine portion and islet cells of the endocrine portion. As the fish grew, the pancreatic tissue began to diffusely distribute around the intestine while also remaining in the visceral face of the liver.

4. Discussion

The organogenesis of the digestive system of *H. platyrhynchos* was studied from hatching to 21 days post-hatching. The hatching occurred 15–17 h after fertilization at 29 °C, which is faster than in other catfish (20–23 h for *Heterobranchus longifilis* at 29 °C (Nwosu and Holzlöhner, 2000), 24–36 h for *Pangasius sutchi* at 20 °C–30 °C (Islam, 2005), 25.5 h for *Rhamdia quelen* at 26 °C (Amorim et al., 2009), 40 h for *Clarias gariepinus* at 24 °C (Osman et al., 2008) and 23 ± 1 h for *Ompok bimaculatus* at 27.0 ± 1.1 °C (Pradhan et al., 2012)). In this study, development was divided into stage I (endotrophic period), stage II (endo–exotrophic period), stage III (exotrophic period without a functional stomach) and stage IV (exotrophic period with a functional stomach) (Table 1 and Fig. 1). After hatching, this species was transparent, and the digestive tract was a straight, undifferentiated tube. According to Andrade et al. (2014), larvae of *H. platyrhynchos* were considered altricial. The exclusively endogenous feeding period was short, and at the end of stage I, this species had an open mouth, an esophagus with longitudinal folds and goblet cells, an incipient stomach and the anterior intestine had a saccular shape. At this time, *H. platyrhynchos* also presented with differentiated structures for finding food, including barbels, eyes and nostrils. The barbels of catfish showed a dense number of taste buds, indicating that gustation plays a primary role in orientation and locating food (Iwai, 1980). The small buccopharynx teeth were also present at this stage, which act to trap prey rather than cut, allowing this carnivorous species to swallow the prey whole.

At 3 DPH, the mouth of *H. platyrhynchos* was open, and they started exogenous feeding. The time of mouth opening varies among species and is influenced by temperature (Gisbert et al., 2004; Zaiss et al., 2006) because temperature increases metabolism (Qu et al., 2012). Moreover, mouth opening and yolk depletion are generally events that are considered to be markers for the beginning of feeding in fish larvae (Gisbert and Williot, 2002). In this studied species, the yolk was only observed until 4 DPH, which is a shorter period than that of other catfish, such as *Clarias gariepinus* (Osman et al., 2008), *Silurus glanis* (Kozarić et al., 2008), *Rhamdia quelen* (Amorim et al., 2009) and *Ompok bimaculatus* (Pradhan et al., 2012), which all have yolks that last 5 days. In this sense, *H. platyrhynchos* presented a short mixed feeding period (endo–exotrophic) of approximately 2 days. In larval rearing, the onset of feeding and the transition of endogenous to exogenous feeding is a crucial moment in developing fish larvae and they have been associated with massive mortalities (Yúfera and Darias, 2007). According to many authors (Chen et al., 2006; Govoni et al., 1986; Segner et al., 1993), the transition of endo–exogenous to exogenous feeding is a critical period because the fish need to develop the ability to survive on solely exogenous feeding. On the other hand, at the end of the endo–exotrophic period and during stage III, *H. platyrhynchos* still did not have a functional stomach, resulting in slower growth than in the period with endogenous resources (Table 1).

Histological analyses of the buccopharynx of *H. platyrhynchos* revealed scattered taste buds at 3 DPH. The taste buds have chemical receptors and mechanoreceptors that increase the efficiency of sorting and selecting food prior to swallowing (Díaz et al., 2009; Northcutt, 2005). The esophagus of *H. platyrhynchos* presented longitudinal folds and an evident tunica muscularis, which enables the distention of the organ and apprehension of prey. The buccopharynx and esophagus had a stratified epithelium with goblet cells that possessed neutral and acidic mucins. According to Galvão et al. (1997), the appearance of goblet cells indicates that the buccopharynx and esophagus are ready to receive exogenous feeding because their secretions protect the epithelium against abrasion and damage caused by the passage of food. Several studies have associated the acidic mucins with the increased viscosity of secretions (Díaz et al., 2008; Tibbetts, 1997) and with lubrication and protection of the epithelium against pathogens and mechanical damage (Fletcher and Grant, 1969; Humbert et al., 1984; Sarasquete et al., 2001). Moreover, neutral mucins are related to the emulsification of food into chyme (Murray et al., 1996) and may indicate pre-gastric digestion (Grau et al., 1992). Thus, the buccopharynx and esophagus of *H. platyrhynchos* contained structures that protected and lubricated the epithelium, indicating that the fish was ready to ingest food at the onset of feeding.

Also at the onset of feeding, the intestine showed a dilated lumen that formed in the anterior region with a saccular shape. This region contained the opening of the duct that secretes bile and pancreatic juice. Many authors have reported larval extracellular proteolytic digestion in the anterior intestine, where the pH is alkaline and trypsin-like enzymes promote proteolytic activity (Gisbert et al., 2004; Walford and Lam, 1993; Zambonino-Infante and Cahu, 2001). In *Pseudosciaena crocea* (Ma et al., 2005), *Salminus brasiliensis* (Vega-Orellana et al., 2006), *Atractoscion nobilis* (Galaviz et al., 2011) and *Huso huso* (Asgari et al., 2013), pancreatic enzyme activities, including trypsin and chymotrypsin, were detected at the first days of the larval stage, emphasizing the importance of these alkaline enzymes during nascent development. *H. platyrhynchos* have a developed liver and extrahepatic pancreas at the onset of feeding, as is observed in adult animals (Faccioli et al., 2014b). Thus, prior to stomach development, the anterior intestine is the primary site of extracellular digestion using pancreatic enzymes and bile juices together with intestinal enzymes.

All intestinal regions of the studied species presented a simple columnar epithelium, although the columnar cells in the anterior intestine were shorter than in the middle and posterior intestine due to the saccular shape of the anterior region. Enterocytes showed supranuclear inclusions primarily in the middle and posterior intestine, which has been similarly observed in many teleosts, including *Paralichthys californicus* (Gisbert et al., 2004), *Plectropomus leopardus* (Qu et al., 2012) and *Thunnus thynnus* (Yúfera et al., 2014). According to Govoni et al. (1986), the existence of acidophilic supranuclear inclusions in enterocytes indicates pinocytotic absorption and intracellular protein digestion during larval developmental. In addition, enterocytes of the studied species showed a PAS-positive brush border. The presence of neutral mucins together with alkaline phosphatases in the intestinal brush border has been correlated to the absorption and transport of macromolecules through membranes (Sarasquete et al., 2001; Stroband et al., 1979).

Goblet cells were visualized throughout the intestine, but they were most numerous in the posterior intestine and were scarce or absent in the rectum. This feature was also observed in adult *H. platyrhynchos* (Faccioli et al., 2014a). Histochemical analyses revealed that goblet cells showed a strong reaction to PAS, whereas staining for AB was increased in the posterior intestine (Table 2). Anderson (1986) associated neutral mucins with supply co-factors required for the enzymatic breakdown of food, and Rhodes et al. (1985) related the acidic mucins to increased resistance of mucus to bacterial degradation and to the absorption of proteins or protein fragments, ions and fluids (Petrinec et al., 2005). The epithelial lining of the rectum exhibited apical secretions composed of neutral and acidic mucins that may be involved in fecal transit, epithelial protection and in the final absorption of substances (Carrassón et al., 2006; Murray et al., 1996).

At 15 DPH, the first gastric glands were observed in the stomach of *H. platyrhynchos*, marking the beginning of stage IV. Generally, gastric glands in fish are composed of a single cell type, oxynticopeptic cells, which are responsible for the production of hydrochloric acid and

pepsinogen (Naguib et al., 2011; Ostos-Garrido et al., 1993). According to Yang et al. (2010), gastric glands in Siluriformes fish appear earlier than in other orders. However, in *H. platyrhynchos*, the gastric glands appeared at 15 DPH, which can be considered a long period as it is longer than that of other catfish, including *Pelteobagrus fulvidraco* at 3 DPH (Yang et al., 2010), *Ompok bimaculatus* at 8 DPH (Pradhan et al., 2012) and *Pangasius sutchi* at 9 DPH (Islam, 2005). The appearance of these glands is an important event in larviculture and, together with pepsin secretion, indicates that the stomach has become functional and marks the transition from larvae to juveniles (Baglole et al., 1997; Ma et al., 2014; Segner et al., 1994). According to Silveira et al. (2013), a morphologically functional stomach and the presence of pepsin-like activity observed in *Rhamdia quelen* 48 h after hatching indicate that the larvae are capable of ingesting and digesting inert food. In *Salminus brasiliensis* larvae, Vega-Orellana et al. (2006) reported that inert food resulted in satisfactory growth only when larvae had a functional stomach. The same was observed in *Clarias gariepinus* and *Scophthalmus maximus* by Segner et al. (1993), concluding that a functional stomach is necessary for utilizing dry food as efficiently as live food. Thus, only with the development of gastric glands at 15 DPH should *H. platyrhynchos* be weaned onto an inert food.

The histochemical analyses revealed that the gastric epithelium had a strong reaction to PAS in the apical region of the epithelial cells (Table 2). This reaction was observed in the majority of teleosts, and it is primarily caused by neutral mucins that are secreted by exocytosis to the gastric lumen (Noaillac-Depeyre and Gas, 1978). According to Ferraris et al. (1987), neutral mucins may protect the epithelium of the stomach against autodigestion by secretions produced by the gastric glands. Moreover, during larviculture of *H. platyrhynchos*, cannibalism was observed. Folkvord and Otterå (1993) reported that cannibalism by differences in size is a major barrier to the intensive farming of carnivorous species. Thus, the larvae must be separated by size into homogeneous size groups.

In summary, this study presents the rarest data on the organogenesis of the digestive system of *H. platyrhynchos*, which is provided to aid in improving the rearing systems of this carnivorous catfish from the Neotropical region. The changes that occur in the digestive system during the first few days are enough to allow for the ingestion and digestion of food, through the appearance of folds and goblet cells in the esophagus, a saccular anterior intestine and development of the liver and pancreas. Feeding occurs at 3 DPH, but gastric glands only appear at 15 DPH. During this period, extracellular digestion occurs in the anterior intestine. Thus, weaning of this species should occur only after the gastric glands appear at 15 DPH.

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