

## Original Article

# Histological description of the larval development of *Brycon gouldingi* Lima, 2004 (Teleostei, Characidae)

Francine Faustino<sup>\*1</sup>, Lilian Cristina Makino<sup>2</sup>, Erika Neumann<sup>3</sup>, Laura Satiko Okada Nakaghi<sup>1</sup>

<sup>1</sup>Centro de Aquicultura da Universidade Estadual Paulista (CAUNESP), Jaboticabal, São Paulo, 14.884-900, Brasil.  
<sup>2</sup>Campus Experimental de Registro, Universidade Estadual Paulista (UNESP), Registro, São Paulo, 11.900-000, Brasil.  
<sup>3</sup>Piscicultura Buriti, Nova Mutum, Mato Grosso, 78.450-000, Brasil.

**Abstract:** Piabanha, *Brycon gouldingi*, is an endemic species in the Tocantins-Araguaia basin. It has aroused the interest of both fish farmers, who started its creation in confinement, and riverine people who appreciate it as a food source. In order to provide information about organic systems of *B. gouldingi* larvae, a histological description was performed after capturing adult specimens in the Rio das Mortes (Mato Grosso, Brazil), adapted to captivity and induced to spawn at Buriti Fisheries (Nova Mutum, MT, Brazil). The collection of samples took place at pre-defined moments after hatching, and the processes relating to morphological differentiation of digestive, excretory, cardiorespiratory, nervous/sensory systems and gas bladder were characterized. At the hatching were found: undifferentiated digestive system; pronephros (primitive kidney), rudimentary heart, central nervous system characterized by primary vesicles, optic vesicle forming the optic cup and crystalline lens. In the course of ontogeny, these organic systems were developed and at the time of the total absorption of the yolk at 55 hours post-hatching were found: the digestive system consisting of the head gut, foregut, midgut and hindgut; two heart chambers and branching of gill arches; three regions of the brain (forebrain, midbrain and hindbrain), neuromasts, olfactory cavity, taste buds; eye consisting of well-defined layers; presence of gas bladder. The results of this study may be useful in providing support for the captive breeding of *B. gouldingi* during the larval stage.

### Article history:

Received 3 October 2017

Accepted 15 April 2018

Available online 25 April 2018

### Keywords:

Ontogeny

Larval development

Organic systems

Light microscopy

## Introduction

The genus *Brycon*, a member of the family Characidae, is widely distributed in South and Central America (Howes, 1982). *Brycon* species are of great importance for Brazilian fish farming, because they have excellent characteristics such as high growth rates and high flavor and quality of the meat for intensive production (Zaniboni-Filho et al., 2006). Piabanha, *B. gouldingi*, is an endemic species of the Tocantins Basin - Araguaia, the largest river basin lying entirely within Brazil (Lima, 2004). This species has a diet based on fruits and insects, living in benthopelagic freshwater environments of tropical climate. They differ from other *Brycon*s for having narrow winding longitudinal stripes (not straight) along the body, darkened pectoral and pelvic fins, distinct V-shaped mark on the caudal fin and caudal

peduncle, and about 66-82 scales in a lateral line (Lima, 2004).

Studies on *B. gouldingi* is rare; in the literature, only the reports of Faustino et al. (2011) which analysed the fertilization and embryonic development of this species based on light and scanning electron microscopy, and Faustino et al. (20015) which conducted the first experiments on the larval stage of the species, with a focus on morphological and morphometric aspects under stereomicroscope can be found. Therefore, research on early development *B. gouldingi* is crucial since it helps fish farmers to improve its reproduction in captivity. In addition, such investigations are urgent because *B. gouldingi* was included in the ordinance of the Ministry of Environment (MMA) No. 445 (17 December 2014) as endangered species classified in endangered category

\*Corresponding author: Francine Faustino  
E-mail address: francine.unesp@gmail.com

(EN) in the list "Brazilian Fauna of Extinction Threatened" (Brasil, 2014).

The larval stage is a limiting factor for *Brycon* farming, considering that the nutritious reserves contained in the yolk sac run out approximately 36 hours post-hatching and larvae start exogenous feeding and cannibalism practices (Oliveira et al., 2004). Success in farming/breeding depends on factors such as quality and quantity of available zooplankton, stocking density of larvae, water quality and homogeneity in the size of the larvae (Ceccarelli and Senhorini, 1996).

The early development of fish is also a critical period in which the differentiation of organ systems occur (Brown and Nuñez, 1994). Nutrition is endogenous during early post-hatching days and once it is depleted, their feeding becomes exogenous; generally, the gastrointestinal tract is not completely developed at this time, leading to morphological constraints, such as mouth size which restricts the number and size of available prey; and physiological constraints, such as incomplete development of digestive glands or incipient enzyme activity, leading to high mortality rate (Govoni et al., 1986).

According to Zavala-Camin (1996), fish larvae generally have similar rudimentary digestive tracts, a single tube format, which undergoes transformations until it reaches the characteristic of adult form. According to Bértin (1958), the digestive tract assumes the following morphological divisions: gut head, which corresponds to the oral cavity and pharynx; foregut, which is equivalent to the segment involving the esophagus and stomach (when present); mid-gut (intestine itself); hindgut, which corresponds to the rectum segment (when present); anus. This terminology corresponds to the morphology of the digestive tract of adults, because fish larvae do not have well-defined segments in their digestive tract during early developmental stages.

Studies related to the ontogenetic development of systems and organs that especially address the digestive system allow identifying the morphological structures related to sorting, capture, digestion and absorption of food, which may assist in evaluating the

factors involved in the mortality of larvae (Maciel, 2006). Hence, this study aimed to provide detailed information regarding larval morphological development of organic systems in piabanha *B. gouldingi* from the hatching upto the total yolk absorption.

## Materials and Methods

**Animals:** Adult piabanha, *B. gouldingi* breeders from Rio das Mortes, Mato Grosso (MT), Brazil were adapted for cultivation in Buriti fish farming, Nova Mutum - MT, Brazil, for about seven months, and selected for induced reproduction according to Woynarovich and Hórvath (1983) between December 2007 and January 2008.

Females and males received common carp, *Cyprinus carpio* pituitary extract to induce spawning, applied to the base of the pectoral fin. In females, the first dose was 0.5 mg.kg<sup>-1</sup> and the second one 5.0 mg.kg<sup>-1</sup> after 10 hours. A single dose of 1.0 mg.kg<sup>-1</sup> was applied to males at the time of the second injection to the females. After stripping, the oocytes were placed in plastic containers and then received the semen, which was lightly homogenized. Then in a few seconds, water was added to the mixture, for activation of gametes and hydration of the eggs, and washed with water to remove excess semen.

The eggs were transported to 200L fiberglass conic incubators with water exchange of 6 L.s<sup>-1</sup>. The water temperature of the incubators was 25.5±0.35°C during the early development of *B. gouldingi*. As an exogenous food source, larvae of the foraging *Leporinus piau* fish was supplied to the *B. gouldingi* larvae at 24 hours post-hatching (hph).

The experiment used a randomized design due to the descriptive nature of the research, which aimed to characterize *B. gouldingi* larvae through microscopic examination. 10 specimens per sampling time were collected: from the hatching larvae (time zero); at every hour up to nine hph; at every 2 hours until 33 hph; and at every 3 hours until the total yolk absorption (55 hph).

This research was approved by the Ethics Committee on Animal Use (0015738-08) and

conducted according to the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Experimentation.

**Light Microscopy:** Samples were fixed in the modified Karnovsky's solution (2.5% glutaraldehyde and 2.5% paraformaldehyde) for 24 hours and then washed and transferred to 0.1 M sodium cacodylate pH=7.4 buffer, and stored under low temperature, being selected and processed for analysis by light microscopy (historesin embedding). Sample processing was performed in the Histology Laboratory of the Morphology and Animal Physiology Department at FCAV-UNESP, Jaboticabal – SP, Brazil.

For historesin embedding (Historesin Plus, Leica, Heidelberg, Germany), the samples were dehydrated for 24 hours in 80% ethanol and then washed twice in 95% and 100% alcohol for 30 minutes each. Afterwards, the samples were stored for 4 hours in a pre-embedding solution consisting of GMA (Glycol Methacrylate) + ethanol (1:1) and 16 hours in the embedding stage (GMA), for further inclusion in histomold. The samples were placed in a drier at 50°C for 24 hours. 2.0 mm semi-seriated histological sections (five sections were discarded) were obtained in a LEICA RM2255 microtome using a tungsten razor. Section staining was performed with Hematoxylin-Phloxine (Tolosa et al., 2003). Slide analysis and photo documentation were obtained using a Leica DM 5000 B light microscope with Leica Application Suite (LAS) software.

## Results

*Brycon gouldingi* larvae hatched 14 hours post-fertilization and total absorption of the yolk happened at 55 hph. By monitoring the larval development, we were able to observe the morphological differentiation processes of the digestive, excretory, cardio-respiratory, nervous/sensory systems and gas bladder.

**Digestive system:** At the hatching, the digestive tract was undifferentiated with an identical epithelium continuous to the integument directly penetrating in the front of yolk sac, marking the future site of the oropharyngeal cavity (Fig. 1A). This epithelium was

sometimes observed as a single layer of cells extending over the yolk sac, and other times as several layers of undifferentiated cells where the intestine differentiates later (Fig. 1B).

The primordia of an oropharyngeal cavity and observation of an oral cleft, marking the location where upper and lower lips would separate later at 1 hph (Fig. 1C). A small lumen was appeared in the digestive tube, at the intestinal region at 2 hph (Fig. 1D), however its posterior part was closed (Fig. 1E). The oropharyngeal cavity was formed at 5 hph, but the lips remained attached and the anterior part of the digestive tube was also closed by a connective tissue (Fig. 1F). The separation of the upper and lower lips and opening of the oropharyngeal cavity were occurred at 9 hph, but the initial portion of the digestive tract remained closed (Fig. 2B), with the first dental alveoli in the pre-maxilla (upper lip) and dental bone (lower lip) being visible (Fig. 2C). The teeth were more elongated at 11 hph (Fig. 2D).

At 13 hph, the digestive tract consisted of the oropharyngeal cavity, esophagus (as a short and narrow tube) and intestine; however connection between gut head and foregut could not yet be identified (Fig. 2E), and the posterior part of the digestive tube was opened (Fig. 2F). It was also possible to identify a portion of the pancreas (Fig. 2E). At 23 hph, the teeth in the lips were more protruding, covered by a simple undefined epithelium (Fig. 3C). At this time, the pleating of the bowel was observed (Fig. 3D). The pharynx was observed at 29 hph, formed by epithelial mucus cells, while the esophagus covered by a simple cylindrical epithelium (Fig. 3E). A small portion of the liver and pancreas was also observed (Fig. 3E). At this time, the digestive tube opened along its entire length. The intestine is dilated with villi and lined by simple cylindrical epithelium (Fig. 3F).

The presence of foraged larvae (supplied *L. piau* at 24 hph) in the gut of *B. gouldingi* larvae was detected at 32 hph, almost occurring from this point onwards, marking the functionality of the digestive tract. At 33 hph, the superficial cells of the pharynx presented cytoplasmic processes protruding inwards and



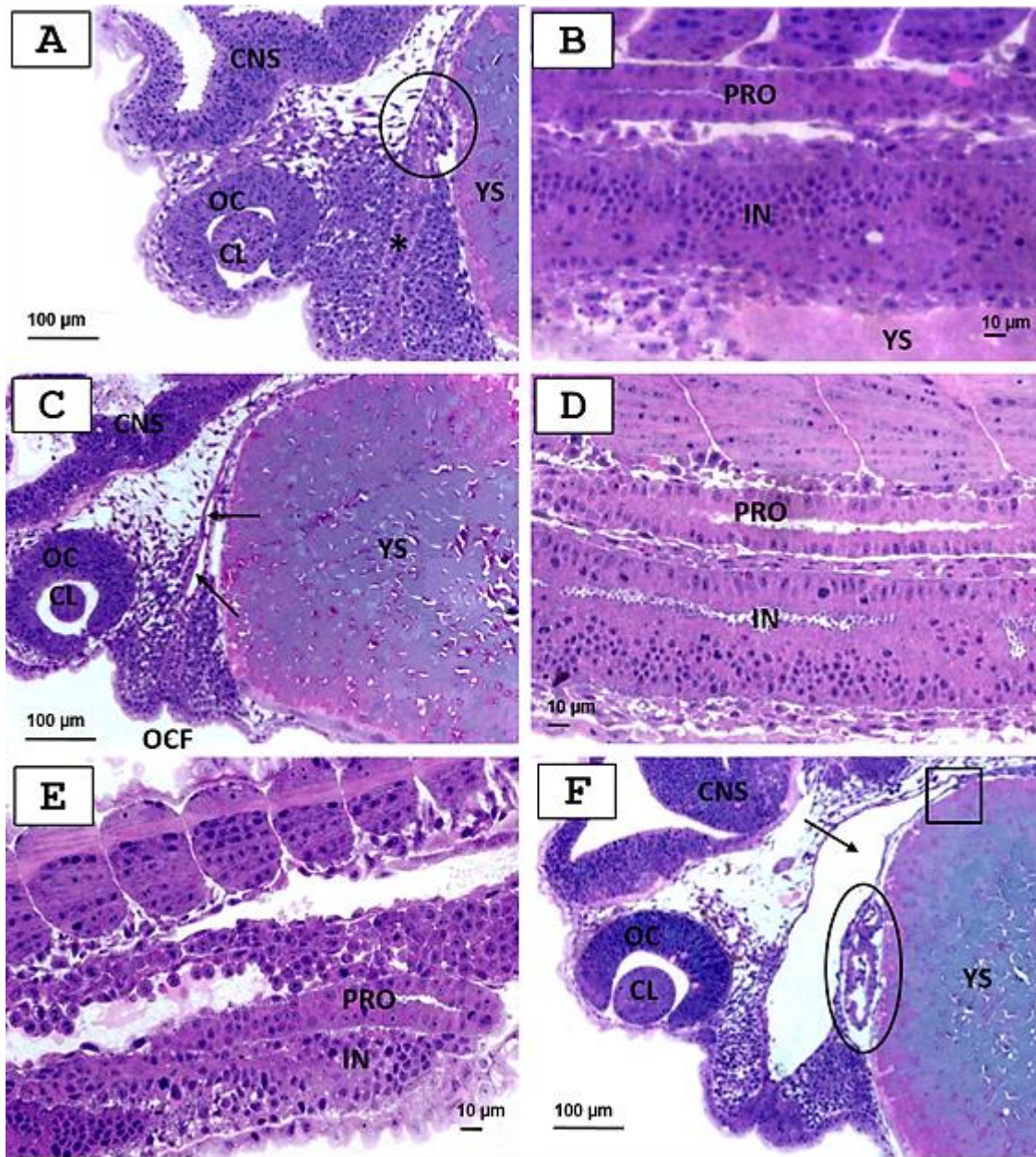


Figure 1. Photomicrographs of *Brycon gouldingi* larvae. **Hatching:** (A) Significant presence of yolk sac (YS); epithelial tegument penetrating and defining the location of the oropharyngeal cavity (asterisk); developing heart (circle) developing eyes with the presence of optic cup (OC) and crystalline lens (CL), (B) pronephro (PRO) and intestine (IN) being defined, **1 hph:** (C) Primitive oropharyngeal cavity (arrow); oral cleft (OCF) marking the site of separation of upper and lower lips; developing central nervous system (CNS) and developing eyes with the presence of optic cup (OC) and crystalline lens (CL), **2 hph:** (D) Pronephro (PRO) and Intestine (IN) with a small visible light (lumen), (E) final portion of pronephro (PRO) and intestine (IN) and **5 hph:** (F) Oropharyngeal cavity developed (arrow); anterior region of the digestive tract closed (square); two chamber heart (circle); developing central nervous system (CNS) and developing eyes with the presence of optic cup (OC) and crystalline lens (CL).

pharyngeal teeth were also observed (Fig. 4C).

The pancreas presented an acinar aspect with basophilic nucleus and acidophilus apical cytoplasm with zymogen granules (Fig. 4D), while liver hepatocytes presented vacuolar cytoplasm presumably due to containing high lipid and glycogen concentrations resulting from the yolk or consumed

larvae (Fig. 4E).

The intestinal epithelial presented brush borders and the presence of digested material noticed in the intestine (Fig. 4F). At this time, the bowel wall was composed of a simple cylindrical epithelium including goblet cells (Fig. 4F). At 36 hph, the dental alveoli were visible on the ceiling of the pharynx that in the



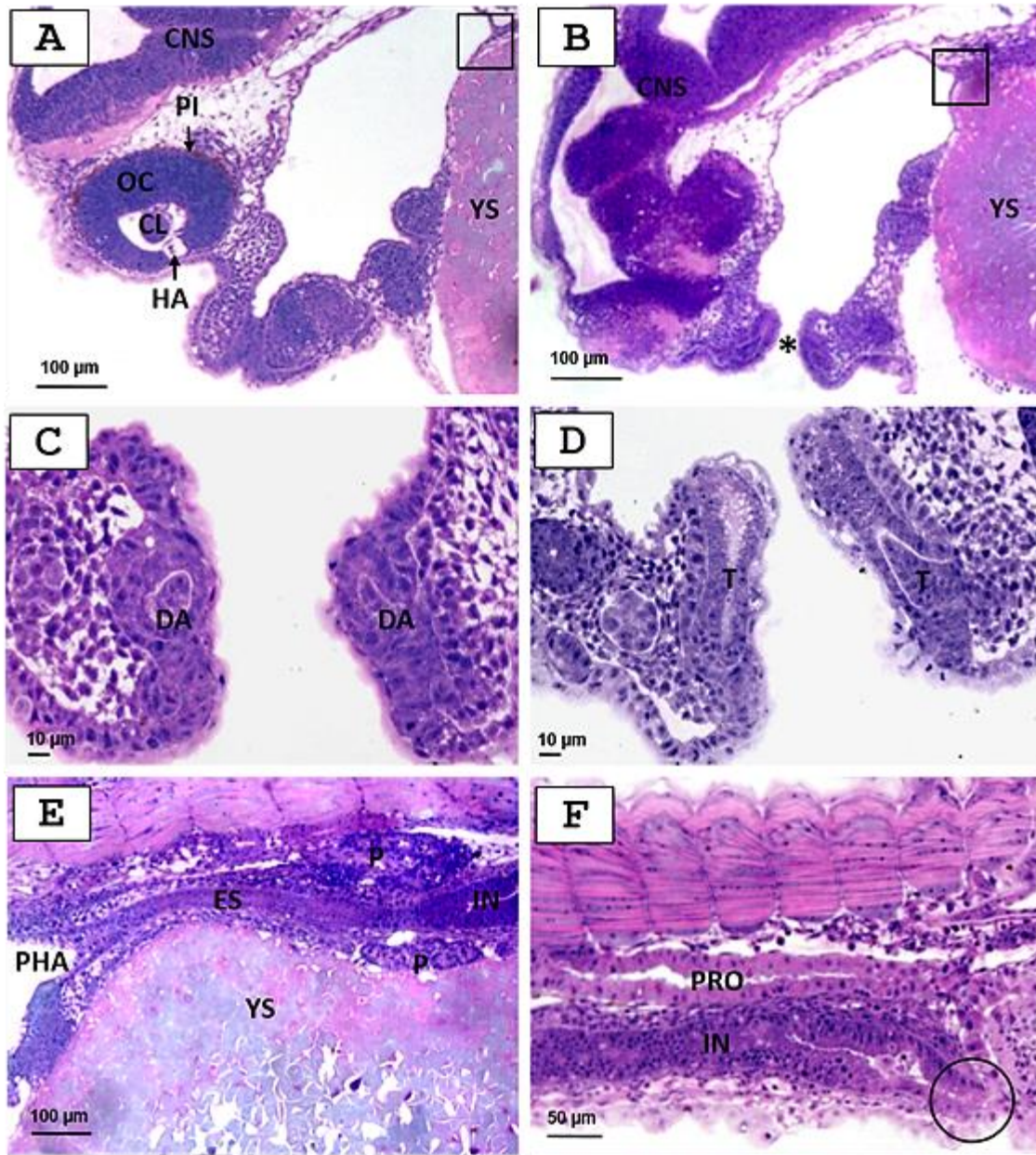


Figure 2. Photomicrographs of *Brycon gouldingi* larvae. **7 hph:** (A) Anterior region of the digestive tract closed (square); developing central nervous system (CNS) and developing eyes with the presence of optic cup (OC), optic vesicle (OV), hyaloid artery (HA) and pigment layer emerging (PI). **9 hph:** (B) Separation of upper and lower lips (asterisk); anterior region of the digestive tract closed (square); developing central nervous system (CNS), (C) dental alveoli (DA), **11 hph:** (D) Primitive teeth (T), **13 hph:** (E) Pharynx (PHA), esophagus (ES), intestine (IN) and pancreas (P) and significant presence of the yolk sack (YS) and (F) pronephro (PRO); end portion of the digestive tract open (circle).

future will originate pharyngeal teeth (Fig. 5B).

Figure 5C shows an ingested forage larva at 39 hph, portraying mixed feeding, since the larvae ingestion occurred before the endogenous reserve (yolk sac) was depleted. Many dental alveoli were present and incisor teeth formed protrusions, indicating that their externalization could happen shortly (Fig. 5D).

At 48 hph, it was possible to identify the septum separating the (actual) midgut and the hindgut (Fig.

6A), digested material (probably consumed forage larva) was found in the midgut leaving it widely dilated (Fig. 6A). At this point, the yolk was almost disappeared. At 55 hph, the yolk sac had already been fully absorbed, and we observed some *B. gouldingi* larvae having more than one foraged larvae within their gut (Fig. 6B).

At this moment the digestive system consisted of the head gut (buccal cavity and pharynx), foregut



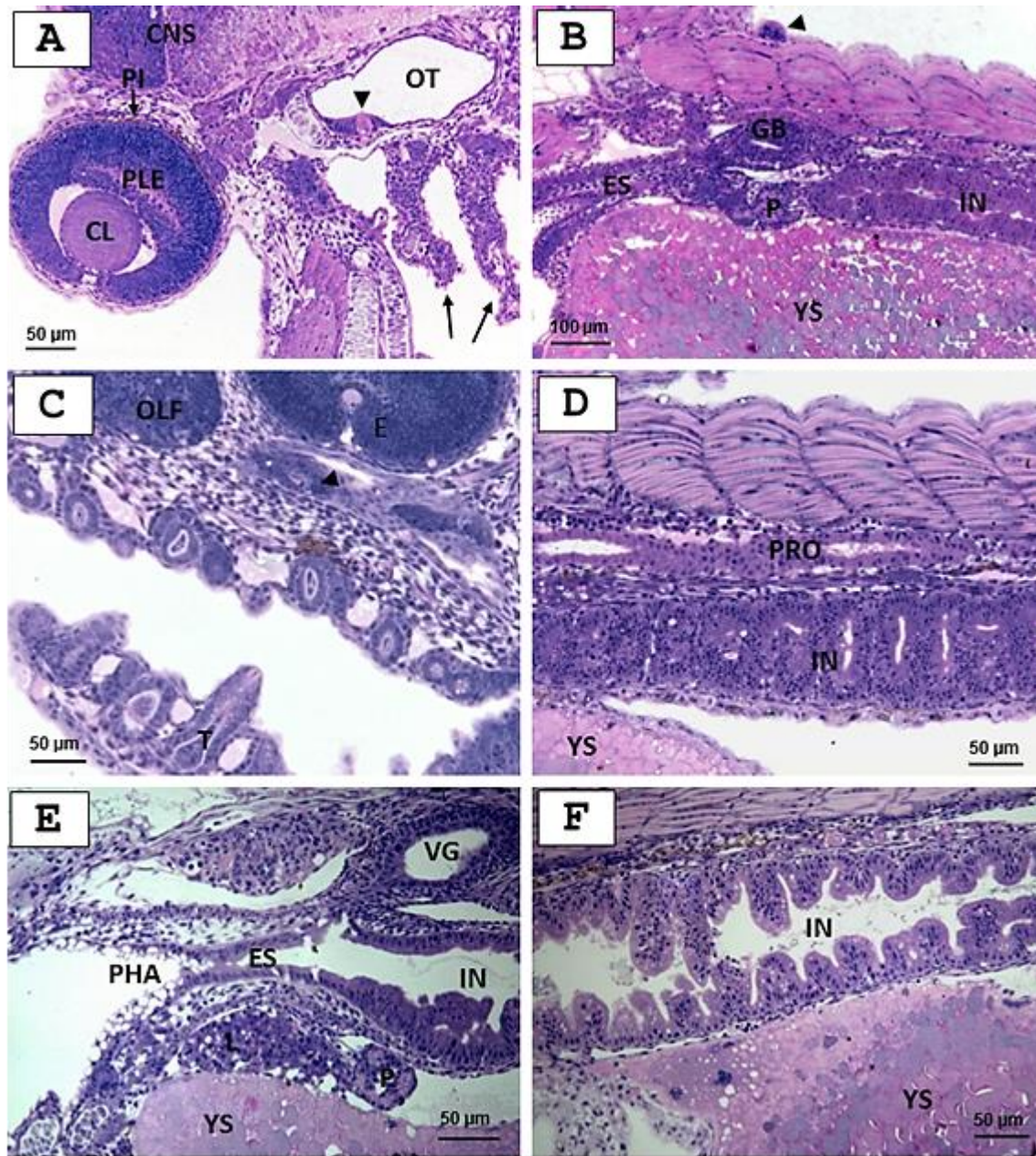


Figure 3. Photomicrographs of *Brycon gouldingi* larvae. **17 hph:** (A) Branching of gill arches (arrow); developing central nervous system (CNS); otic vesicle (OT) with neuromasts (arrowhead); developing eyes with the presence of well-formed crystalline lens (CL), pigment layer (PL) and plexiform (PLE) emerging, **21 hph:** (B) Esophagus (ES); intestine (IN); pancreas (P); superficial neuromast (arrowhead); gas bladder (GB); yolk sac (YS), **23 hph:** (C) Primary teeth (T); neuromast (arrowhead) near the eyes (E); presence of olfactory cavity (OLF), (D) pronephro (PRO); intestine circumvolutions (IN), **29 hph:** (E) Pharynx (PHA); esophagus (ES); intestine (IN); liver (L); pancreas (P); gas bladder (GB) and (F) middle portion of the intestine dilated (IN).

(represented by the esophagus), midgut (gut itself) and hindgut. The esophagus showed no significant changes in its structure throughout this study. Most teeth remained covered by the epithelium, while many dental alveoli and some exteriorized teeth were observed.

During the study period, there was also no differentiation of the stomach or gastric glands which

probably occur later. Differentiation of the rectal portion and the anus was also not verified.

**Excretory system:** The pronephros (primitive kidney) observed from the hatching, just above the gut, following the entire length thereof and consisting of a simple cylindrical epithelium (Fig. 1B). A small lumen appears in the pronephros at 2 hph (Fig. 1D, E). With larval development at 39 hph, the cranial portion



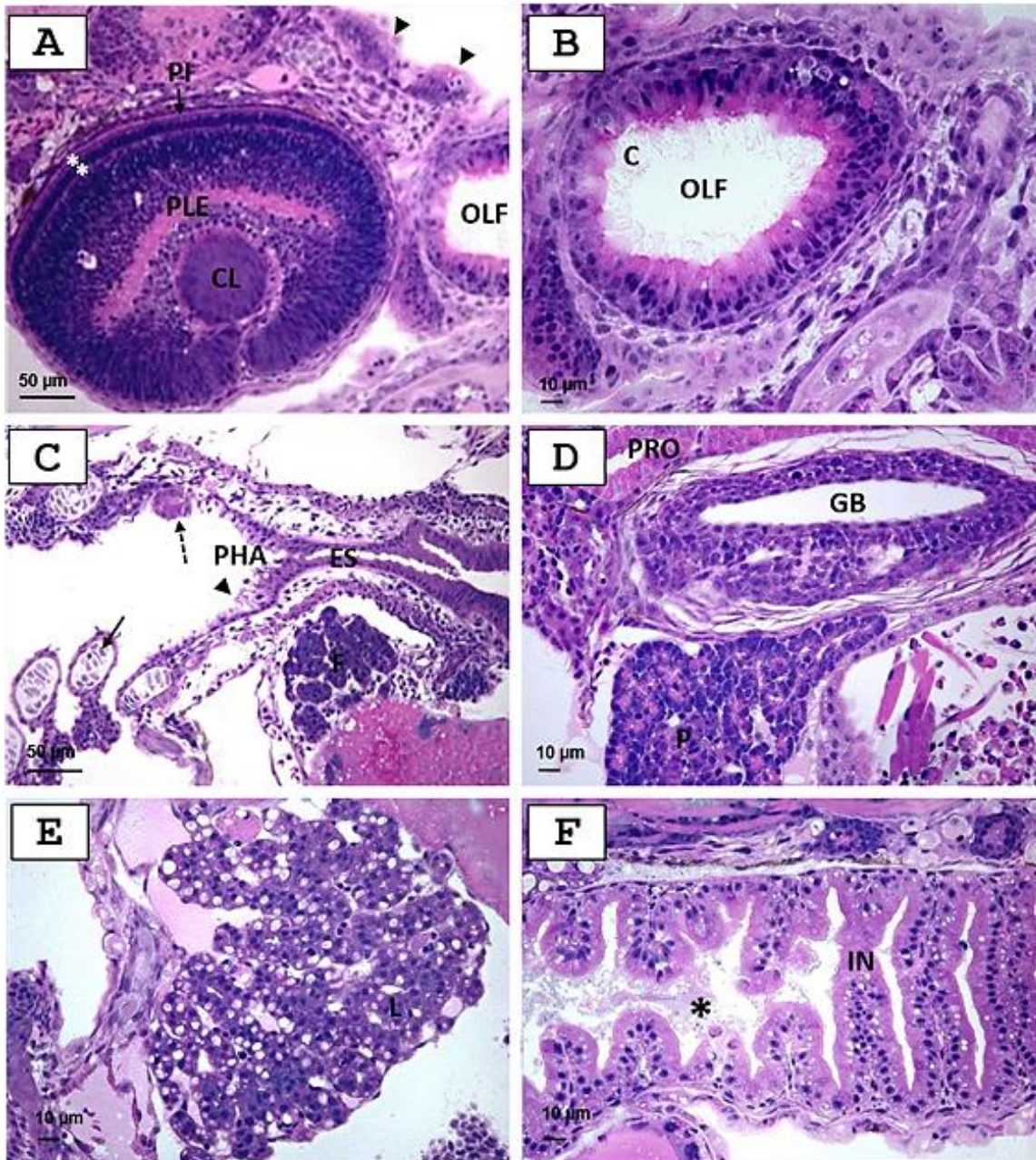


Figure 4. Photomicrographs of *Brycon gouldingi* larvae. **31 hph:** (A) Neuromasts (arrowhead); well-formed crystalline lens (L); pigment layer (PL) and photoreceptor layer (two asterisks) developing; evident plexiform layer (PLE); olfactory cavity (OLF), (B) olfactory cavity with cilia (OLF), **33 hph:** (C) Pharynx (PHA) with cytoplasmic processes (star), esophagus (ES); liver (L); gill arches (arrow); primary pharyngeal teeth (dotted arrow), (D) gas bladder (GB); pancreas (P); cranial portion of pronephro coiling (PRO), (E) liver (L) and (F) digested material (asterisk) in the intestine (IN).

of the pronephros located in the dorsal part of the body cavity, above the gas bladder, becomes coiled and its lumen is more apparent (Fig. 5E), while the caudal region remains straight (Fig. 5C).

**Cardiorespiratory system:** A rudimentary heart is observed in the pericardial cavity, anterior to the yolk sac and the abdominal cavity at hatching (Fig. 1A). Two heart chambers (atrium and ventricle) were

observed at 5 hph (Fig. 1F). The branching of gill arches was detected at 17 hph (Fig. 3A).

**Nervous/Sensory system:** Development of the central nervous system was characterized by initially being presented as primary vesicles at hatching (Fig. 1A). At this moment, the optic vesicle (first embryonic stage of the eye) had already invaginated, forming the optic cup, as well as the crystalline lens (Fig. 1A).



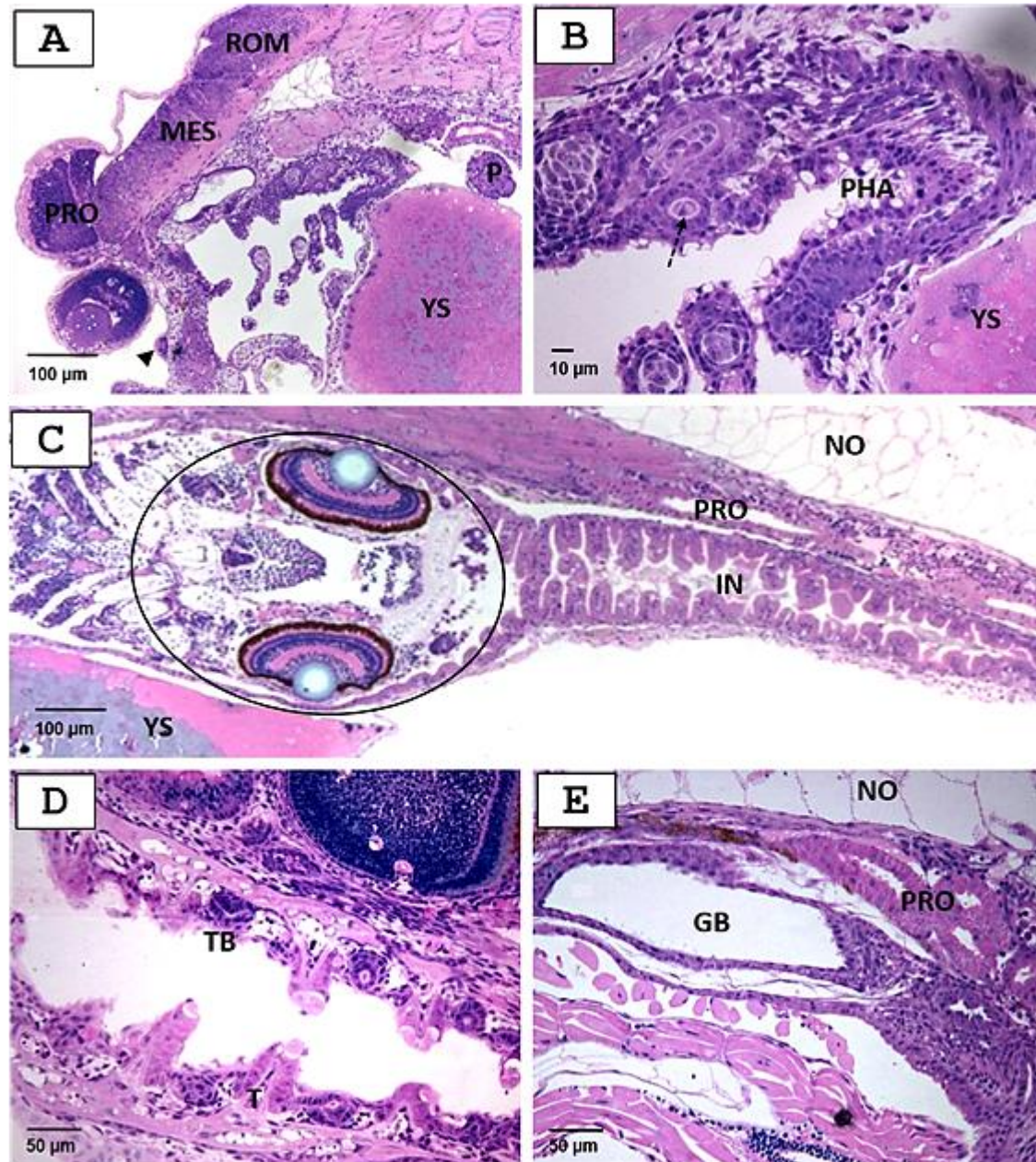


Figure 5. Photomicrographs of *Brycon gouldingi* larvae. **36 hph:** (A) Well-defined prosencephalon (PRO), mesencephalon (MES) and rhombencephalon (ROM); neuromasts (arrowhead); yolk sac (YS); pancreas (P), (B) presence of dental alveolus (dotted arrow) on the roof of the pharynx (PHA); yolk sac (YS), **39 hph:** (C) ingestion of forage larva (circle); notochord evident (NO); Pronephro (PRO); intestine (IN); yolk sac (YS), (D) tooth (T); taste buds (TB) and (E) partially inflated gas bladder (GB); cranial portion of the Pronephro coiled (PRO).

At 7 hph, the hyaloid artery and pigment layer appeared in the peripheral region of the optic cup (Fig. 2A). At 9 hph, development of the central nervous system was evidenced by observing the cells of the primitive vesicles filling out (Fig. 2B).

The eye lens was well-formed at 17 hph, and the plexiform layer began to emerge (Fig. 3A). Larvae showed free neuromasts inside the otic vesicle (Fig. 3A) and also throughout the body surface in the

cephalic region (Fig. 3B).

At 23 hph, several neuromasts were observed near the orbital optical vesicles (Fig. 3C). At 31 hph, the plexiform layer of the eye was better visualized, and the double layer of photoreceptors could also be distinguished, with surface neuromasts (Fig. 4A).

The olfactory cavity was composed of the ciliated pseudostratified cylindrical epithelium (Fig. 4B). The central nervous system was well-developed at 36 hph,



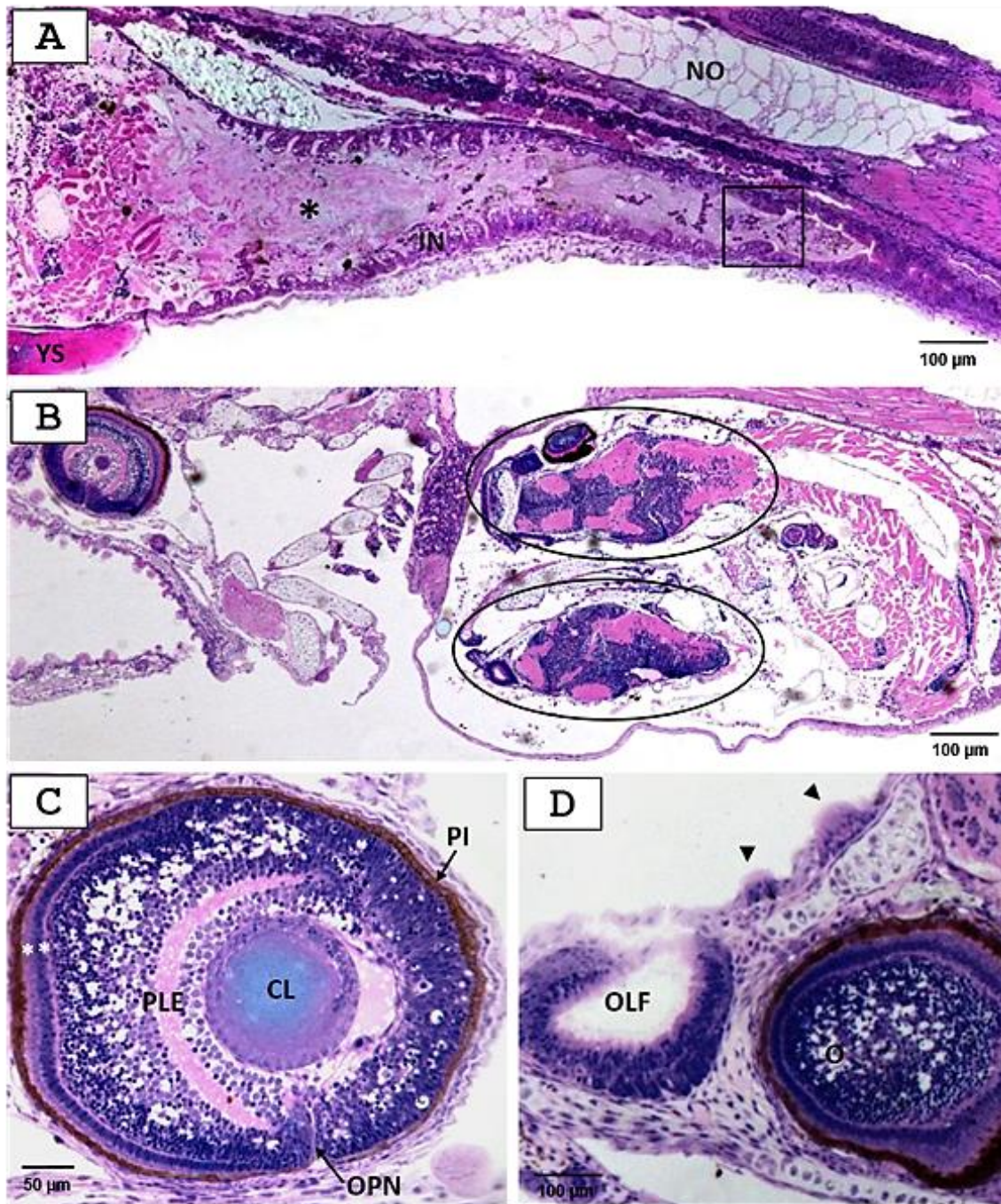


Figure 6. **Photomicrographs of *Brycon gouldingi* larvae.** **45 hph:** (A) Digested material (asterisk) in the intestine (IN); notochord evident (NO); small remaining portion of the yolk sac (YS). **55 hph:** (B) Presence of forage larvae (circles) in the intestine; septum between middle and hindgut (Square). (C) Well-developed eyes with well-formed crystalline lens (L); pigment layer (PL), plexiform layer (PLE), photoreceptor layer (two asterisks) and evident optic nerve (OPN). (D) olfactory cavity (OLF); neuromasts (arrowhead) around the eyes (E).

when the three regions of the brain prosencephalon (forebrain), mesencephalon (midbrain) and rhombencephalon (hindbrain) were distinct (Fig. 5A). The notochord was well characterized at 39 hph, (Fig. 5C and 5E) extending throughout the body length of the larvae. The cells showed vacuolar cytoplasm. The presence of taste buds was also noticed (Fig. 5D).

The eye was well-developed at 55 hph, consisting of well-defined lens, pigment layers (outer) and well prominent plexiform, in addition to the dual layer of photoreceptors (Fig. 6C). Several neuromasts were seen around the eye orbital (Fig. 6D).

**Gas bladder:** At 21 hph, the formation of the gas bladder began, and connected to the esophagus by a

connector duct (Fig. 3B). It was formed and covered by a stratified squamous epithelium at 33 hph (Fig. 4D), located below the notochord and above the digestive tract. At 39 hph, it was partially inflated (Fig. 5E).

## Discussion

In most fish species, newly hatched larvae have simple, undifferentiated digestive tracts (Govoni et al., 1986). Indeed at the time of hatching, *B. gouldingi* presented a rudimentary digestive tract, while at the time of total yolk absorption the digestive tract divided into gut head, foregut, midgut and hindgut, the same classification adopted by Bértin (1958).

Rodrigues et al. (2006) have stated that the mouth, oral cavity and pharynx, which constitute the head gut, are associated to the capture, guidance, and pre-digestive preparation of food. They also suggest that the thicker lips and oral teeth help to hold food, which in omnivorous species are used for pre-digestive preparation of plant food material, and to capture and hold animal prey. The pharyngeal teeth observed in *B. gouldingi* assist in capturing and macerating soft-bodied organisms.

When exogenous feeding was initiated, the mucus cells were also found in the pharynx of *B. gouldingi*, and according to Galvão et al. (1997), these cells participate in gliding prey. The presence of mucous cells was also observed in the esophagus, indicating that along with the development of the folds in the esophagus, which precede the differentiation of the stomach, these cells facilitate the rapid transition of food to the posterior segment (González et al., 2002; Chen et al., 2006; Yang et al., 2010).

Until the total yolk absorption, the esophagus in *B. gouldingi* showed no significant differentiation. According to Zavala-Camin (1996), the esophagus in fish is a tubular body that serves as a passageway between the oral-pharyngeal cavity and the stomach; and in the case of physostomous species and similarly in this study, with the pneumatic duct opening of the gas bladder in the esophagus.

The formation of the stomach and gastric glands was not observed in *B. gouldingi* until the total yolk

absorption. In that period, according to Watanabe and Kiron (1994), fish depend on the ability to select food, mechanical digestion, and pancreatic and intestinal enzymes which act in an alkaline medium to compensate for the absence of gastric enzymes.

The efficiency of digestion and absorption of food is due to the presence of folds and microvilli in the intestines, considering that a mature liver and pancreas help in the digestion of food for producing digestive secretions which digest proteins, fats and sugars (González et al., 2002). A differentiated liver and pancreas are usually observed in larvae that had already begun exogenous feeding soon after hatching and which are functional before the yolk is completely absorbed; a fact confirmed in *Paralabrax maculofasciatus* (Peña et al., 2003), *Seriola lalandi* (Chen et al., 2006), *Pelteobagrus fulvidraco* (Yang et al., 2010), and also found in this study. According to González et al. (2002), the presence of folds in the intestine, as observed in *B. gouldingi*, indicate an increase in efficiency of food digestion and absorption. Seixas-Filho et al. (2000) have suggested that mucosal transverse folds slow food passage, allowing for a greater digestive period and better utilization of nutrients in the midgut, as this type of arrangement does not occur in the hindgut. Goblet cells were also observed in the midgut portion and, as reported by George et al. (1998), these cells secrete basic mucus, with the function to neutralize stomach acid and prepare the food bolus for the action of pancreatic and intestinal enzymes that act in a basic medium.

Regarding the development of the excretory system, the pronephros was present from the hatching of *B. gouldingi* larvae, and according to Kimmel et al. (1995), it developed in the early stage of somitogenesis. According to Drummond et al. (1999), pronephros only differentiate into mesonephro in youth/adult individuals.

The heart is the first organ to develop and become functional during embryogenesis (Hu et al., 2000). In *B. gouldingi*, the heart was rudimentary at the hatching; it was located in the pericardial cavity anterior to the yolk sac and the abdominal cavity,



similarly to what has been reported in newly hatched larvae from other studies (Hu et al., 2000; Falk-Petersen, 2005).

According to Bone et al. (1995), most teleost larvae hatch performing gas exchanges through the skin; this type of breathing is appropriate since many of these larvae are transparent and inhabit pelagic regions, where oxygen is plentiful and the required hemoglobin could make them visible to predators. However, with the growth of the larvae, the cutaneous respiration becomes inefficient, being performed through the gills. The protuberance from which the gill arches will emerge was visualized in *B. gouldingi* soon after hatching, and became well-developed after the total yolk absorption. Leonardo et al. (2001) reported that gills are vital structures for fish health, since they are the main site for gas exchanges, as well as also being involved in osmoregulation processes, acid-base balance and excretion of nitrogenous compounds.

The first sensory structures to be visualized in *B. gouldingi* larvae were the eyes, olfactory cavity, then the otic vesicle and neuromasts, while taste buds were the last to be identified, as reported by Uyan et al. (2006) for *Eugraulis japonicus* larvae. During larval development, the eye is the sensory organ that plays a key role in detection of prey and escape from predators (Moorman, 2001). The eye development in *B. gouldingi* happened gradually. In the larvae when yolk was absorbed, the eye was developed in the last moment of observation and had its main layers formed and the nuclei of the rod and cone cells were starting to form two layers, similarly to larvae of *Oreochromis niloticus* (Morrison et al., 2001). The hyaloid artery found in this study irrigated the optic cup and the crystalline lens under development. Vision is critical to fish survival, especially after the start of exogenous feeding, since most species are considered visual consumers, as well as to avoid predators (Carvalho et al., 2004). Nascimento et al. (2015) showed that feeding behavior of *Betta splendens* larvae was particularly dependent visual ontogeny, since the larvae were able to capture *Artemia* more efficiently with the progress of development, when there was a

greater eye movement of larvae in the presence of food.

The olfactory plate was present in *B. gouldingi* from the hatching, corroborating reports by Hansen and Zeiske (1993), which stated that the differentiation of the olfactory plate in embryos and fish larvae is fast; however, the process is slow until complete formation. The olfactory organs act as chemoreceptors to capture the smell of the saturated substances in the water and consequently, the food supply (Matsuoka, 2001). The neuromasts are mechanoreceptors superficially located in the skin, which can be presented as free neuromasts and/or grooves and channels, forming the lateral line system. In *B. gouldingi*, neuromasts are observed in the lateral line, but they were also observed close to the eye sockets.

According to Gilbert (2010), the anterior portion of the neural tube undergoes dramatic changes and expands into three primary vesicles: forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon). In larvae of *B. gouldingi*, the central nervous system has been completely filled observed for cells and delimited in the three portions at 36 hph. The development of the central nervous system is related to the onset of aggressive behavior in fish larvae such as cannibalism (Sakakura and Tsukamoto, 2002).

The gas bladder, observed as an outline in *B. gouldingi* located in the dorsal part of the body, as other fishes above the center of gravity of the fish allowing them to maintain posture without relying on muscular effort (Hidelbrand, 1995). Godinho et al. (2003) reported that the rise of gas bladder (along with the emergence of the pectoral fins) are remarkable events of larval ontogeny, since they facilitate the balance and direction in the water column.

Based on the results, it can be concluded that the development of *B. gouldingi* was quick, a common characteristic among species of freshwater teleost, with simultaneous differentiation of structures that allow capturing of food early in the development before endogenous energy reserves run out, and also ensuring they can escape predators, increasing the

chance of survival during the larval stage. This information is unprecedented for the *B. gouldingi* species and indispensable for farmers who already produce or intend to start farming of *B. gouldingi*, thus contributing to better performance and production in captivity.

### Acknowledgments

The authors thank Buriti Fisheries (Nova Mutum, MT, Brazil) for providing the eggs and fish and to O. Mateus, histo-technician from the Histology and Embryology Laboratory of FCAV/UNESP, Jaboticabal-SP, Brazil, for his help in material processing; F.F. acknowledges FAPESP for the award of a Masters Scholarship (2007/57826-7) and CNPq (473712-2007-5) for financial assistance.

### References

- Bértin L. (1958). Appareil digestif. In: P.P. Grassé (Ed.). *Traité du Zoologie*. Paris: Masson. pp: 1249-1301.
- Bone Q., Marshall N.B., Blaxter J.H.S. (1995). *Biology of fishes*, 2nd edition. Blackie, London. 332 p.
- Brasil, Ministério Do Meio Ambiente, Portaria MMA (2014). Reconhece como espécies de peixes e invertebrados aquáticos da fauna brasileira ameaçadas de extinção aquelas constantes da "Lista Nacional Oficial de Espécies da Fauna Ameaçadas de Extinção - Peixes e Invertebrados Aquáticos". Diário Oficial da União, Brasília, DF, 17 de dezembro de 2014.
- Brown C.L., Nuñez J.M. (1994). Hormones: Actions and applications in embryogenesis. In: K.G. Davey, R.E. Peter, S.S. Tobe (Eds.). *Perspectives in Comparative Endocrinology*. Ottawa, National Research Council. pp: 333-339.
- Carvalho P.S.M., Noltie D.B., Tillit D.E. (2004). Biochemical, histological and behavioral aspects of visual function during early development of rainbow trout. *Journal of Fish Biology*, 64: 833-850.
- Ceccarelli P.S., Senhorini J.A. (1996). *Brycon*: viabilização da produção de alevinos. *Panorama da Aquicultura*, 6: 10-11.
- Chen B.N., Qin J.G., Kumar M.S., Hutchinson W., Clarke S. (2006). Ontogenetic development of the digestive system in yellow tail kingfish *Seriola lalandi* larvae. *Aquaculture*, 256: 489-501.
- Drummond I.A., Majumdar A., Hentschel H., Elger M., Solnika-Krezel L., Schier A.F., Neuhauss S.C.F., Stemple D.L., Zwartkruis F., Rangini Z., Driever W., Fishman M.C. (1999). Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. *Development*, 125: 4655-4667.
- Falk-Petersen I.B. (2005). Comparative organ differentiation during early life stages of marine fish. *Fish and Shellfish Immunology*, 19: 397-412.
- Faustino F., Makino L.C., Neumann E., Nakaghi L.S.O. (2015). Morphological and morphometric aspects of early life stages of piabanha *Brycon gouldingi* (Characidae). *Journal of Fish Biology*, 86: 1491-1506.
- Faustino F., Nakaghi L.S.O., Neumann E. (2011). *Brycon gouldingi* (Teleostei, Characidae): aspects of the embryonic development in a new fish species with aquaculture potential. *Zygote*, 19: 351-363.
- Galvão M.S.N., Fenerich-Verani N., Yamanaka N., Oliveira I.R. (1997). Histologia do sistema digestório da tainha *Mugil platanus* (Günther, 1880) (Osteichthyes, Mugilidae) durante as fases larval e juvenil. *Boletim do Instituto de Pesca*, 24: 91-100.
- George L.L., Alves C.E.R., Castro R.R.L. (1998). *Histologia comparada* (2nd edition). São Paulo, Roca. 286 p.
- Gilbert S.F. (2010). *Developmental Biology*. (9th edition). Sunderland MA, Sinauer Associates. 685 p.
- Godinho H.P., Santos J.E., Santos Y. (2003). Ontogênese larval de cinco espécies de peixes do São Francisco, In: H.P. Godinho, H.L. Godinho (org.). *Águas, peixes e pescadores do São Francisco das Minas Gerais*. Belo Horizonte, PUC Minas. pp: 133-148.
- González O.R.M., Flores J.C.B., Domínguez B.M.P., Valle M.R.G. (2002). Descripción histológica del sistema digestivo em larvas de *Chirostoma humboldtianum* em la primera alimentación exógena. I Congreso Iberoamericano Virtual de Acuicultura. Civa 2002. (<http://www.civa2002.org>). pp: 313-322.
- Govoni J.J., Bohler G.W., Watanabe Y. (1986). The physiology of digestion in fish larvae. *Environmental Biology of Fish*, 16: 59-77.
- Hansen A., Zeiske E. (1993). Development of the olfactory organ in the zebrafish, *Brachydanio rerio*. *Journal of Comparative Neurology*, 333: 289-300.
- Hidelbrand M. (1995). Análise da estrutura dos vertebrados. São Paulo: Atheneu. 700 p.
- Howes G. (1982). Review of the genus *Brycon* (Teleostei, Characoidei). *Bulletin of the British Museum (Natural History)*, 43: 1-47.



- Hu N., Sedmera D., Post H.J., Clark E.B. (2000). Structure and function of the developing zebrafish heart. *The Anatomical Record* 260: 148-157.
- Kimmel C.B., Ballard W.W., Kimmel S.R., Ullmann B. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203: 253-310.
- Leonardo J.M.L.O., Vargas L., Ribeiro R.P., Moreira H.L.M., Natali M.R.M., Volski T., Cavichiolo F. (2001). Histologia das brânquias de larvas de tilápia do Nilo, *Oreochromis niloticus* (L.) de origem tailandesa submetidas a diferentes níveis de vitamina C. *Acta Scientiarum*, 23: 863-870.
- Lima F.C.T. (2004). *Brycon gouldingi*, a new species from the rio Tocantins drainage, Brazil (Ostariophysi: Characiformes: Characidae), with a key to the species in the basin. *Ichthyological Exploration of Freshwaters*, 15: 279-287.
- Maciel C.M.R.R. (2006). Ontogenia de larvas de piracanjuba, *Brycon orbignyanus* Valenciennes (1849) (Characiformes, Characidae, Bryconinae). Tese (Doutorado em Zootecnia). Universidade Federal de Viçosa, Viçosa. 229 p.
- Matsuoka M. (2001). Development of sense organs in the Japanese sardine *Sardinops melanostictus*. *Fisheries Science*, 67: 1036-1045.
- Moorman S.J. (2001). Development of sensory system in zebrafish (*Danio Rerio*). *ILAR Journal*, 42: 292-298.
- Morrison C.M., Miyake T., Wright J.R. (2001). Histological study of the development of the embryo and early larva of *Oreochromis niloticus* (Pisces: Cichlidae). *Journal of Morphology*, 247: 172-195.
- Nascimento N.F., Valentin F.N., Santos M.P., Chavarro S.Y.C., Manzini B., Paes M.C.F., Faustino F., Silva R.C., Nakaghi L.S.O. (2015). Initial Feeding Behaviour, Eye structure and effect of colours on prey capture rates of *Betta splendens* larvae. *Journal of Fisheries and Aquatic Science*, 10: 357-366.
- Oliveira A.M.B.M.S., Conte L., Cyrino J.E.P. (2004). Produção de Characiformes autóctones. Cap. 8. In: J.E.P. Cyrino, E.C. Urbinati, D.M. Fracalossi, N. Castagnolli (Eds.). *Tópicos especiais em piscicultura de água doce tropical intensiva*. São Paulo: TecArt; Sociedade Brasileira de Aquicultura e Biologia Aquática. pp: 217-238.
- Peña R., Dumas S., Villalejo-Fuerte M., Ortíz-Galindo J.L. (2003). Ontogenetic development of the digestive tract in reared spotted sand bass *Paralabrax maculatofasciatus* larvae. *Aquaculture*, 219: 633-644.
- Rodrigues S.S., Navarro R.D., Menin E. (2006). Adaptações anatômicas da cavidade bucofaringeana de *Leporinus macrocephalus* Garavello & Britski, 1988 (Pisces, Characiformes, Anostomidae) em relação ao hábito alimentar. *Biotemas*, 19: 51-58.
- Seixas-Filho J.T., Brás J.M., Gomide A.T.M., Oliveira M.G.A., Donzele J.L., Menin E. (2000). Anatomia funcional e morfometria dos intestinos e dos cecos pilóricos do Teleostei (Pisces) de água doce *Brycon orbignyanus* (Valenciennes, 1849). *Revista Brasileira de Zootecnia*, 29: 313-324.
- Tolosa E.M.G., Behmer A.O., Freitas Neto A.G. (2003). Manual de técnicas para histologia normal e patológica. São Paulo: Edart, Edusp. 240 p.
- Uyan S., Kawamura G., Vazquez-Archdale M. (2006). Morphology of the sense organs of anchovy *Engraulis japonicus*. *Fisheries Sciences*, 72: 540-545.
- Watanabe T., Kiron V. (1994). Prospects in larval fish dietetics. *Aquaculture*, 24: 223-251.
- Woyanovich E., Horváth L. (1983). A propagação artificial de peixes de águas tropicais: manual de extensão. Brasília: FAO/CODEVASF/CNPq. 225 p.
- Yang R., Xie C., Fan Q., Gao C., Fang L. (2010). Ontogeny of the digestive tract in yellow catfish *Pelteobagrus fulvidraco* larvae. *Aquaculture*, 302: 112-123.
- Zaniboni-Filho E., Reynalte-Tataje D., Weingartner M. (2006). Potencial del género *Brycon* en la piscicultura Brasileña. *Revista Colombiana Ciencias Pecuarias*, 19: 233-240.
- Zavala-Camim L.A. (1996). Introdução ao estudo sobre alimentação natural de peixes. Maringá, EDUEM. 129 p.