Received: 15 August 2015 | Accepted: 13 May 2016

DOI: 10.1111/jai.13127

ORIGINAL ARTICLE

WILEY Journal of Applied Ichthyology DWK

Development of the digestive tract in first feeding larvae of *Betta splendens* Regan, 1910

R. C. Silva[1] | F. N. Valentin[1] | M. C. F. Paes[1] | F. Faustino[1] | L. G. Giannecchini[1] | P. H. O. Viadanna[1] | N. F. Nascimento[1] | J. B. K. Fernandes[1] | L. S. O. Nakaghi[1,2]

[1]Aquaculture Centre (CAUNESP), Universidade Estadual Paulista, Sao Paulo, Brazil

[2]Laboratory of Histology and Embryology, Department of Morphology and Animal Physiology, School of Agrarian Sciences and Veterinary Medicine, Universidade Estadual Paulista, Sao Paulo, Brazil

Correspondence
Laura S. O. Nakaghi, Aquaculture Centre (CAUNESP), Universidade Estadual Paulista, Sao Paulo, Brazil.
Email: laurankg@fcav.unesp.br

Summary

The aim of this study was to analyse the development of the digestive tract of Siamese fighting fish larvae (*Betta splendens* Regan, 1910), from hatching to 92 hours post-hatching (hph) at 28.0°C, in order to determine the most appropriate time to begin exogenous feeding (live feed) and to investigate the digestive tract and its function during larval development. At hatching (29 hours post-fertilization), the digestive tract was a simple and straight undifferentiated tube lined by a single layer of columnar epithelial cells; the mouth and anus were closed. At 18 hph, the mouth was open. At 32 hph, the tongue was located in the depression of the buccopharyngeal cavity floor and supported by basal cartilage. Goblet cells were present in the epithelium lining the pharynx and oesophagus. At 56 hph, the midgut had four distinct layers: the mucosa with typical villi, the submucosa, the smooth muscle layer, and the serosa. Histological analysis revealed that the larvae retained endogenous yolk reserves until 74 hph. Lipid accumulation was observed in the liver, which coincided with complete yolk absorption. At this time, the digestive tract was fully open and functional. Thus, it can be concluded that live feed should be given to *B. splendens* larvae from 74 hph onward, when the larvae are able to consume the food provided. This study also provides useful data for the improvement of husbandry techniques and for the formulation of diets specific to ornamental fish larvae.

1 | INTRODUCTION

Betta splendens Regan 1910, originated in Southeast Asia (Thailand, Malaysia), and is known as the Siamese fighting fish due to an aggressive behaviour between the males (Giannecchini, Massago, & Fernandes, 2012). It is reared in captivity worldwide as an aquarium fish for its favourable characteristics such as a broad variety of colours, long and wide fins, and the presence of an accessory respiratory organ that allows it to be kept in small tanks with no aeration (Zuanon, Salaro, Veras, Tavares, & Chaves, 2009). Because of its popularity and high profitability, *B. splendens* is an important species in the ornamental fish market and industry (Chapman, Fitz-Coy, Thunberg, & Adams, 1997).

Larviculture is one of the most important and limiting stages in fish production. Similarly limiting is the period of transition from endogenous (yolk) to exogenous feeding (live feed), which is marked by high mortality rates and low production efficiency due to great histomorphological changes in the digestive tract (He et al., 2012; Papandroulakis, Mylonas, Maingot, & Divanach, 2005; Støttrup & Norsker, 1997; Wootton, 1990).

Successful transition feeding, and the consequential productive larviculture, requires that all tissues and organs related to capture, ingestion and absorption of the food be functional and that the appropriated food is available at the right moment, insuring survival and growth of the fish larvae (Kjørsvik, Pittman, & Pavlov, 2004; Yúfera & Darias, 2007). Larvae of most fish species have relatively low enzymatic activity, low hydrolysis, and nutrient absorption capacity; thus, the supply of live feed is essential during this stage as it aids digestion by providing digestive enzymes from the body of the prey (Kolkovski, 2001). However, the first step in understanding the ontogeny and function of the digestive tract in fish is to promote morphological

studies (He et al., 2012; Micale, Garaffo, Genovese, Spedicato, & Muglia, 2006; Nakatani et al., 2001).

In order to contribute to the design of nutritional studies and to the improvement of rearing technologies for *B. splendens* larvae, the aim of this study was to analyse the morphological and histological structure of the digestive tract during its ontogeny, from hatching to 92 hours post-hatching (hph) and thus determine the most appropriate time to begin exogenous feeding.

2 | MATERIALS AND METHODS

2.1 | Broodstock, reproductive management and sample collection

Sixty mature *B. splendens* (30 males, 30 females) were individually kept in 2-L plastic containers for 10 days, to acclimate to test conditions. Standard length (mean SL ± *SD*) of males was 3.68 ± 0.04 cm and weight 1.82 ± 0.2 g. For females, SL was 3.49 ± 0.18 cm and weight 1.63 ± 0.04 g, respectively. Fish were fed ad libitum with *Artemia* sp. nauplii twice daily.

The plastic containers with the breeders were kept in a water bath inside polyethylene boxes. Water temperature was maintained at 28.0 ± 0.2°C by four 200-watt heaters, and a submerged 2,000 L hr^{-1} BOYU pump used to circulate the water between the boxes. The boxes were covered with polyethylene lids equipped with 60 cm "daylight" 20-watt fluorescent lamps that provided 500-lux (Lutron Light Meter, model LX-105) within each experimental box. The lighting system was automatically turned on and off by analogical timers (Giannecchini et al., 2012) set to provide a photoperiod of 12 hr light:12 hr dark. Partial exchange of water was performed daily, during daytime.

Water temperature, dissolved oxygen and pH levels of the plastic containers housing the adult fish and larvae were monitored daily (means ± *SD*): temperature 28.0 ± 0.2°C, dissolved oxygen 5.3 ± 0.2 mg L^{-1} (oximeter YSI model 55) and pH 6.9 ± 0.3 (pH meter YSI, model PH100). Ammonia concentration was determined weekly and at a mean concentration of <0.1 mg L^{-1} recorded throughout the experiment (CELM spectrophotometer, Model E-225D).

After acclimatization, 20 females with the most prominent reproductive signs (i.e. salient urogenital papilla) were selected and transferred to individual transparent plastic cups (200 ml) with holes, which were placed in the containers of reproductively viable males (presence of bubble nests) for 24 hr. After this period, the females were release into the container. If the couple did not exhibit normal mating behaviour within 15 min (i.e. if the male was aggressive towards his partner), the couple was separated and the female returned to her container. After 24 hr, the breeders were observed every 10 min until the male wrapped his body around the female and the eggs were released.

Eight couples spawned simultaneously and, in order to reduce genetic interference, the spawns were pooled together and then divided into eight separate 2-L plastic containers, with 220 larvae each. The males were kept with the eggs until they hatched, for parental care. Exogenous feed was given to *B. splendens* larvae twice a day, from 48 hph, using *Artemia* sp. nauplii as live feed.

2.2 | Sampling

Sample collection began when the majority of larvae (>50%) had hatched, at 29 hours post-fertilization (hpf; time zero). Samples were collected every 2 hr up to 20 hph, then every 4 hr up to 44 hph, and subsequently every 6 hr up to 92 hph. Larvae were randomly selected from the plastic containers and each larva was considered a replica.

2.3 | Morphometry and stereomicroscopy analysis

The larvae were euthanized by immersion in benzocaine solution (250 mg L^{-1}) and observed under a LEICA MZ8 stereomicroscope equipped with a LEICA DFC 280 camera, using the IM 50-LEICA software.

Total length (TL) in millimetres (mm) and body weight in micrograms (μg) of the *B. splendens* larvae (n = 20) from each sampling time, from 29 hpf to 92 hph, were recorded and analysed using the statistic program SAS 9.1 (SAS Institute, Cary, NC, USA). Data are expressed as mean ± standard deviation (*SD*).

Thirty larvae were randomly selected from 48 to 92 hph and analysed under a stereomicroscope (LEICA MZ8) to determine the percentage of those with food in their digestive tracts.

2.4 | Histological analysis

For light microscopy analysis (n = 6 at every collection), half of the samples (n = 3) were fixed in 4% buffered formalin, washed in 0.1 mol L^{-1} phosphate buffer (pH 7.4), and dehydrated in a series of increasing ethanol concentrations before being cleared in xylene and embedded in Histosec®. Samples were serially sectioned (5 μm) and stained with Haematoxylin-Eosin (HE; Tolosa, Rodrigues, Behmer, & Freitas Neto, 2003). The other half of the samples (n = 3) were fixed in modified buffered Karnovsky's solution (paraformaldehyde + glutaraldehyde), washed in 0.1 mol L^{-1} phosphate buffer, dehydrated in 80% ethanol for 24 hr followed by three washes of 30 min in 90%, 95% and 100% ethanol before being embedded in historesin (Leica). The samples were serially sectioned (2.0 μm) and stained with Haematoxylin-Phloxine (HP) for histological analysis (Tolosa et al., 2003). The sections were analysed and photographed under a Leica DM 2500 microscope using the Leica Application Suite (LAS) software.

2.5 | Ultrastructural analysis

For scanning electron microscopy analysis, samples (n = 4 at every collection) were fixed in modified buffered Karnovsky's solution, transferred to sodium cacodylate buffer, post-fixed in 1.0% osmium tetroxide for 2 hr, washed in buffer and dehydrated in a series of increasing ethanol concentration solutions. Samples were then subjected to critical point drying with liquid CO_2, mounted on copper

holders and coated with gold-palladium. Electron photomicrographs were obtained with a JEOL-JSM 5410 scanning electron microscope.

3 | RESULTS

This study adopted the classification proposed by Bértin (1958): the digestive tract of *Betta splendens* was divided into the cephalic gut (oral cavity and pharynx, including the lips), foregut (oesophagus and stomach), midgut (intestine) and hindgut (rectum and anus).

Larval hatching (>50%) occurred at 29 hpf and the larvae presented (mean ± *SD*) body weight of 213.00 ± 15.66 μg (n = 20) and total length (TL) of 2.26 ± 0.23 mm (n = 10). Mean larval body weight and total length, from hatching to 92 hph, are summarized in Table 1. While the standard length showed a linear increase with time, a reduction in body weight was observed at the time of complete yolk sac absorption, which coincided with the feeding transition, at 74 hph.

TABLE 1 Mean ($\bar{X}$) ± standard deviation (*SD*) of total length in millimeter (mm) and body weight in micrograms (μg) of *B. splendens* larvae, from hatching (time zero) to 92 hours post-hatching (hph), n = 20

	Body weight (μg)		Total length (mm)	
hph	$\bar{X}$	±*SD*	$\bar{X}$	±*SD*
0	213.20	42.80	2.26	0.23
2	214.10	27.94	2.46	0.03
4	214.10	15.24	2.51	0.09
6	214.00	13.14	2.58	0.09
8	214.70	36.07	2.62	0.08
10	216.10	33.51	2.71	0.07
12	215.80	28.89	2.73	0.10
14	217.10	11.36	2.74	0.11
16	218.00	10.33	2.81	0.10
18	218.20	16.96	2.82	0.09
20	219.00	17.20	2.85	0.06
24	221.40	14.01	2.84	0.09
28	223.80	21.18	2.90	0.13
32	228.20	19.84	2.94	0.06
36	232.00	30.67	2.95	0.11
40	239.00	30.69	2.98	0.11
44	251.30	26.49	3.06	0.10
50	257.40	30.05	3.10	0.08
56	268.70	36.19	3.18	0.16
62	282.60	28.20	3.25	0.07
68	273.30	44.93	3.26	0.10
74	312.00	25.61	3.29	0.16
80	341.30	17.86	3.47	0.18
86	377.60	101.23	3.51	0.18
92	431.80	55.78	3.65	0.17

Newly-hatched larvae had their heads attached to the anterior portion of the yolk sac, were non-pigmented, showed distended posture and had no fins, except for an embryonic fin enveloping the entire caudal region. The larvae hatched with an externally closed mouth and anus. The digestive tract appeared as a straight tube (rudimentary intestine) with a single layer of columnar cells and lay dorsally to the yolk sac with a slightly expanded anterior portion (Fig. 1a).

Early development of the buccopharyngeal cavity was observed at 2 hph, represented by two undifferentiated cell layers (data not shown). At 4 hph, the hindgut was partially open and, at 6 hph, the midgut primordium appeared as a small cleft (Fig. 1b).

At 8 hph, scanning electron microscopy revealed the early formation of the mouth as an oral groove (Fig. 4a). At 10 hph, the swim bladder was lined with vacuolated columnar epithelium cells (Fig. 1b); the buccopharyngeal cavity remained closed by a membrane (Figs 1b,bI and 4b). In the coelomic cavity, the rudimentary intestine was formed by a single layer of columnar epithelium, with medial or basal nuclei in the ventroposterior region (Fig. 1bII). The endodermal cells began to differentiate into hepatocytes to form the liver. The primordial swim bladder was differentiated from the dorsal wall of the digestive tract, appearing as an oval chamber; however, neither a pneumatic duct nor the rete mirabile were distinguishable at this time (Fig. 1b).

At 14 hph, a small lumen lined with simple columnar epithelium could be observed in the mid-region of the digestive tube (Fig. 1c), from where the intestinal segment originated. The branchial arches were visible (data not shown) and the embryonic kidney (pronephros) began to differentiate dorsally to the intestine (Fig. 1c). The anus was open by 16 hph (Fig. 4b – detail).

At 18 hph the mouth was open, with non-pigmented lower and upper lips (Fig. 4c). The lower lip was slightly thicker and exhibited greater mobility than the upper lip. Cilia could be seen near the mouth (Fig. 4c). The superior and inferior oral valves began to develop at the anterior end of the buccopharyngeal cavity. The branchiostegal membrane, which later would cover the branchial arches, also began to form at this time.

At 20 hph, a protuberance appeared on the epithelium of the buccopharyngeal cavity and would later become the tongue. A pneumatic formation connected the swim bladder to the digestive tract. The gallbladder was spherical, with squamous epithelium, and could be seen close to the liver. The pancreas was located externally to the hepatic tissue (Fig. 1e).

At 32 hph, the tongue was situated in the depression of the buccopharyngeal cavity floor and was supported by a basal cartilage (Fig. 1d). A small number of melanophores was observed on top of the tongue and taste buds were seen on the epithelial lining (Fig. 1d). No pharyngeal teeth are present in this species. The digestive tract was open from the mouth to the anus (Fig. 1d,e); a pneumatic duct was observed running from the digestive tract, near the junction of the oesophagus and stomach, to the posterior end of the swim bladder that was not yet inflated (Fig. 1e). The exocrine pancreas, which was located between the stomach, liver and intestine (Fig. 1e), consisted of clusters of pyramidal cells mostly organized in acini. These cells showed acinar expression pattern, had a dark basophilic cytoplasm,

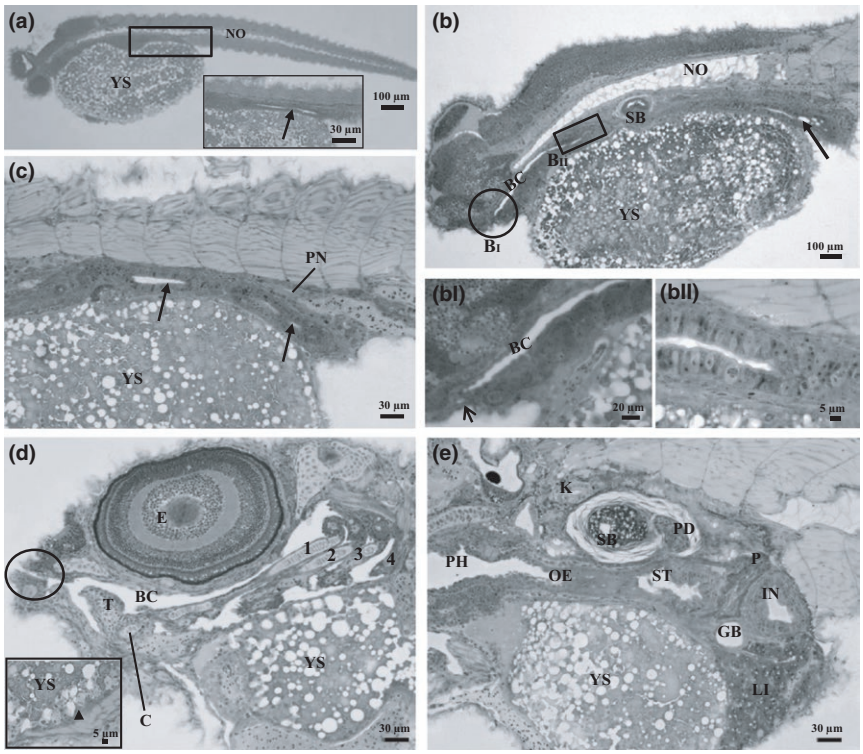

FIGURE 1 Histology of the digestive tract in *B. splendens* larvae from hatching to 32 hours post-hatching (hph) (HP staining). (a) Newly hatched larvae. Detail: a straight undifferentiated tube lined by a single layer of columnar epithelial cells. (b) 10 hph: the differentiated primordial swim bladder could be seen as an oval chamber. The buccopharyngeal cavity was closed by a membrane (circle, see detail in BI). There was a cleft on the anterior region of the digestive tube, which was formed by a simple layer of columnar epithelium, with medial or basal nuclei (rectangle, see detail in BII). (c) 14 hph: mid-region lumen of the digestive tube, emergence of the pronephros and final portion of the hindgut. (d) 32 hph: the eyes were well developed and the lips (circle) were separated. In the buccopharyngeal cavity, the tongue was supported by basihyal cartilage and very evident branchial arches mainly composed of cartilage (1, 2, 3 and 4). Detail showing the syncytial layer (arrowhead) absorbing the yolk, note the reduction on the size of the yolk sac. (e) 32 hph: Some structures that compose the digestive tract were already differentiated but not yet functional, such as: pharynx, oesophagus, stomach, intestine, swim bladder (not inflated) with pneumatic duct, kidney, liver, gallbladder, pancreas and kidney. BC, branchial arches (1, 2, 3 and 4); buccopharyngeal cavity; C, cartilage; E, eye; GB, gallbladder; IN, intestine; K, kidney; LI, liver; NO, notochord; OE, oesophagus; P, pancreas; PD, pneumatic duct; PH, pharynx; arrow, primitive gut; PN, pronephros; SB, swim bladder; ST, stomach; T, tongue; YS, yolk sac; and HP, Haematoxylin-Phloxine

distinct nuclei, and several large eosinophilic zymogen granules that increased in number until total yolk absorption (Fig. 2f).

From 32 to 40 hph, there was a saccular dilation in the stomach at the posterior region of the oesophagus (Fig. 2e). At 36 hph, the swim bladder was inflated. At 40 hph, the buccopharyngeal cavity was lined with simple squamous epithelium, with goblet cells present in the epithelium lining the buccopharyngeal cavity, pharynx region and oesophagus (Fig. 3a). Small teeth, aligned in a single row, were present in the maxilla (Fig. 4d). Neuromasts were distributed near the mouth, along the body and on the head (Fig. 4e). By 44 hph, the secondary lamellae of the branchiae were fully developed (Fig. 3b).

At 56 hph, four basic layers could be seen in the midgut (intestine): (i) mucosa, containing typical villi and lined with a pseudostratified columnar epithelium (enterocytes) with brush border and lamina propria consisting of connective tissue, (ii) submucosa, consisting of loose connective tissue, (iii) smooth muscle layer, containing longitudinal and transverse bundles, and (iv) outer serosa, consisting of loose connective tissue and a simple squamous epithelium. In the distal portion of the midgut, numerous goblet cells could be seen between the enterocytes on the brush border of the mucosa (Fig. 3g,h). The hindgut mucosa (rectum and anus) contained deep folds that projected into the lumen, and was lined with simple columnar epithelium and goblet cells (Fig. 3g,h). The hindgut submucosa and serosa layers were similar to those observed in the midgut; the muscle layer, however, was thicker in the hindgut.

At 68 hph, the oesophagus lumen had longitudinal folds and was lined with a simple columnar epithelium (Fig. 2c) consisting of goblet cells interspersed with mucosal cells. Four well-defined layers could be observed: (i) mucosa, with a simple columnar epithelium and lamina propria, (ii) submucosa, consisting of loose connective tissue, (iii) large muscle layer, composed of striated skeletal muscle, and (iv) outer serosa covering the organ (Fig. 2c). The difference in the muscle layer of the oesophagus and the stomach was clearly visible, enabling the identification of the transition between these organs (Fig. 2e). The muscular layer varied considerably throughout its development.

The stomach had a saccular shape and was formed by four layers: the mucosa with deep rounded folds, the submucosa, a thick muscular layer, and the serosa. At this time, remnants of the yolk sac were still

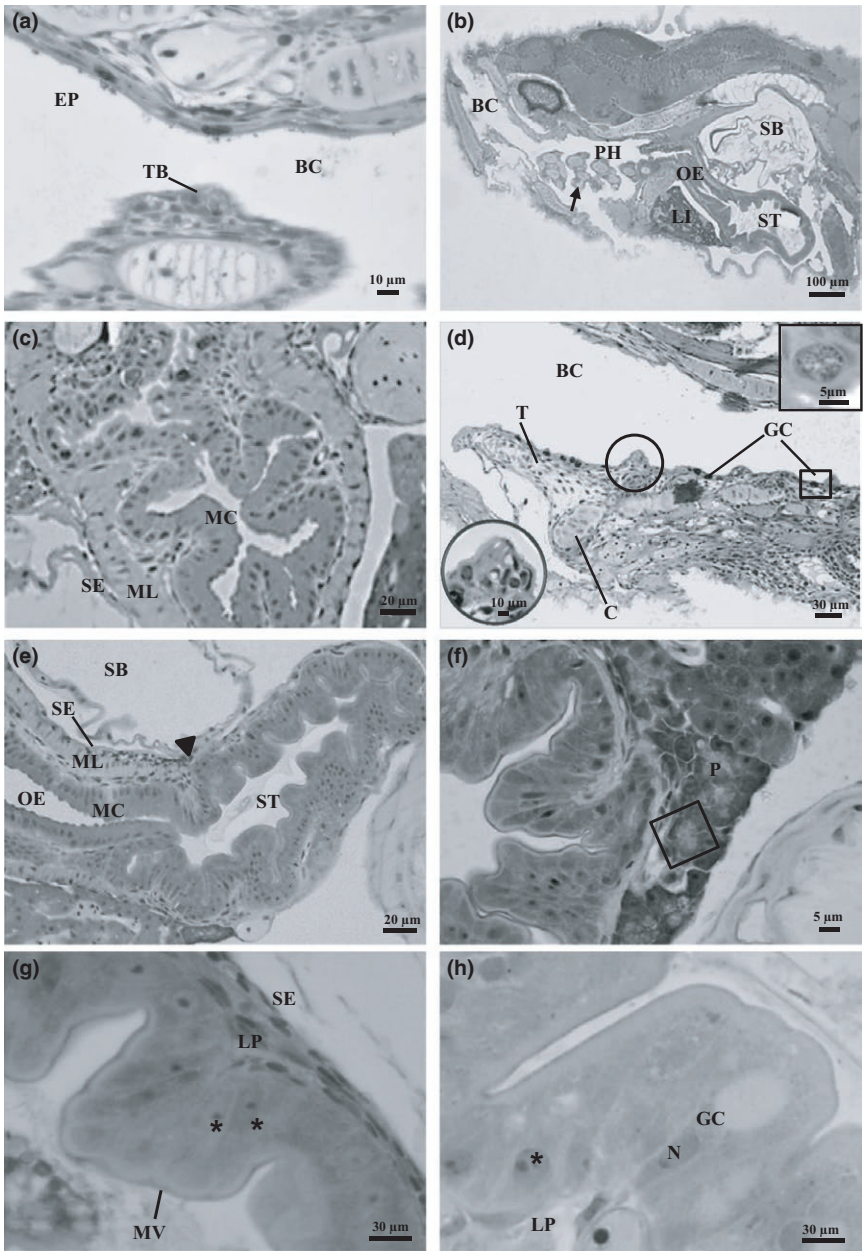

FIGURE 2 Histology of the digestive tract in *B. splendens* larvae from 56 hours post-hatching (hph) to 86 hph (HP staining). (a) 56 hph: the buccopharyngeal cavity was lined by simple squamous epithelium and contained a taste bud. (b) 56 hph: main view of the digestive tract and branchia with secondary lamellae (arrow). Note the tongue with basal cartilage, short oesophagus containing folds, and inflated swim bladder. (c) 68 hph: transversal section of the oesophagus with folds of the mucosa, muscle layer and serosa. Note the thickness of the muscle layer. (d) 80 hph: buccopharyngeal cavity with taste buds and goblet cells; the tongue is supported by basihyal cartilage. Note the large number of goblet cells lining the epithelium covering the buccopharyngeal cavity. Detail: taste buds (circle) and goblet cell (rectangle). (e) Digestive tract showing the transition region from the oesophagus (arrowhead) to the stomach. (f) Pancreatic acini (highlighted). (g) 86 hph: intestinal mucosa showing microvilli. (h) 86 hph: intestinal mucosa with scattered goblet cells (with basal nucleus) between enterocytes (*). BC, buccopharyngeal cavity; closed arrow, branchial lamellae; C, cartilage; EP, epithelium; GB, gallbladder; GC, goblet cell; LP, lamina propria; LI, liver; MC, mucosa; ML, muscle layer; MV, microvilli; N, goblet cell nuclei; OE, oesophagus; P, pancreas; PH, pharynx; SE, serosa; SB, swim bladder; ST, stomach; TB, taste bud; T, tongue; and HP, Haematoxylin-Phloxine

present and, in the liver, polyhedral hepatocytes were arranged along hepatic sinusoids with no sign of lipid deposition. Stereomicroscopic analysis revealed that at 68 hph only a few larvae (10%) were able to ingest exogenous feed. At 74 hph, the liver showed large lipid storage vacuoles within the hepatocytes (Fig. 3a) and food could be seen in the stomach and intestine in the majority (>70%) of the larvae, which coincided with complete yolk absorption. At 80 hph, hepatic lipid deposition was noticeably reduced (Fig. 2b) and absent by 86 hph (Fig. 2c).

At 80 hph, the upper oral valve was wider than the lower valve (Fig. 4e). Teeth were inserted in the inner part of the upper and lower labial borders and showed a smooth surface without cusps. The mandibular teeth were larger and more numerous than the maxillary teeth. At 92 hph, pointed teeth could be seen (Fig. 4f) and successive changes in the position of the mouth, from ventral to dorso-terminal, were observed.

It was also observed that the structures involved in locomotion (swim bladder and fins) and sensory organ (eyes and neuromasts) were formed simultaneously to the digestive tract.

4 | DISCUSSION

Hatched larvae possess a rudimentary and undifferentiated digestive tract and are thus fully dependent on an endogenous source of nutrition. In the present study, the mouth of the larvae opened at 18 hph; however, exogenous feeding began concomitantly to the depletion of the yolk sac, at 74 hph. In Hoplias lacerdae larvae, the yolk sac is almost completely absorbed by 192 hph (Maciel, Maciel, Lanna, & Menin, 2009), which demonstrates that the best time to start exogenous feeding varies among species. Prior to the opening of the mouth,

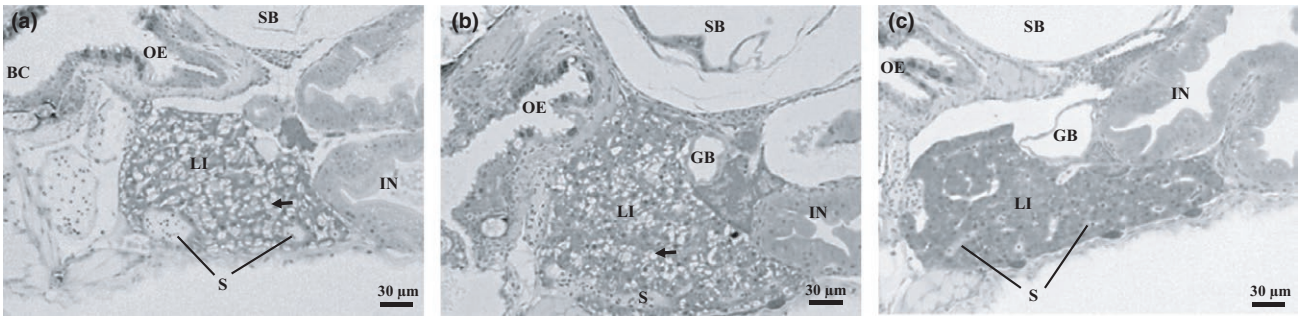

FIGURE 3 Histology of the liver in *B. splendens* larvae at different stages of development (HE staining). (a) 74 hph: liver with large lipid storage vacuoles within hepatocytes (arrow), augmented hepatic sinusoids and more intrahepatic vacuoles. (b) 80 hph: liver with lipid storage vacuoles. (c) 86 hph: hepatocytes arranged along hepatic sinusoids and without lipid deposition. arrow, lipid storage vacuoles; GB, gallbladder; LI, liver; S, hepatic sinusoids; SB, swim bladder; and HE, Haematoxylin-Eosin

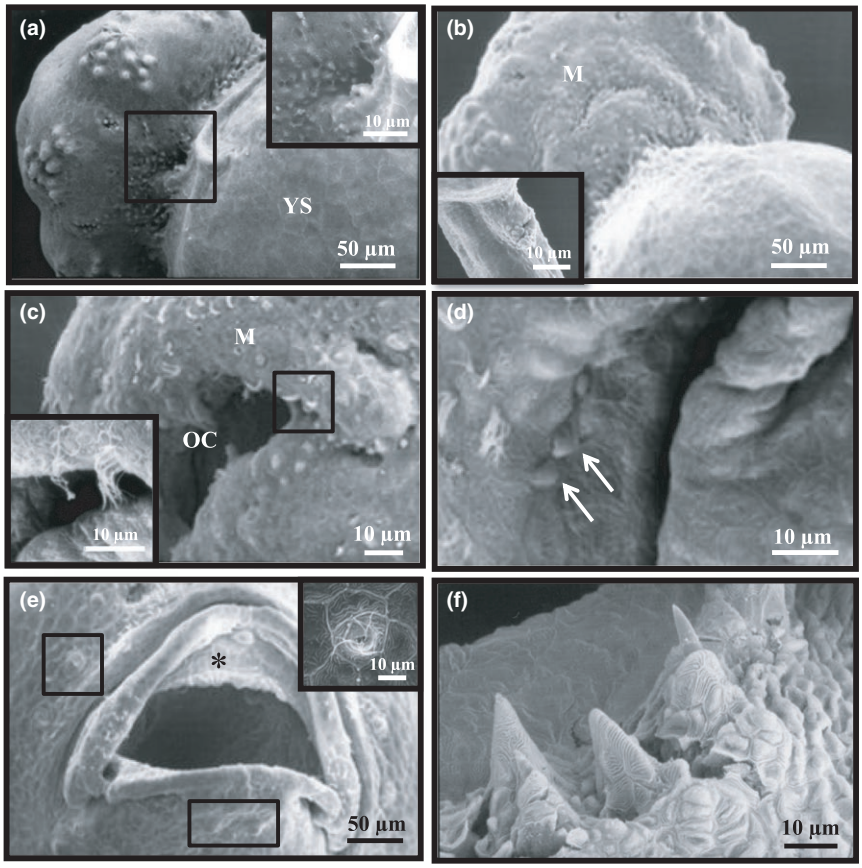

FIGURE 4 Scanning electron photomicrographs of *Betta splendens* during larval development. (a) 8 hph: a groove (detail) developed on the anterior region of the head, forming the early mouth. (b) 16 hph: mouth being developed (detail: open anus). (c) 18 hph: open mouth. Note presence of cilia on the upper lip (detail). (d) 40 hph: denticles in the maxilla. (e) 80 hph: superior valve in the mouth (*). Note presence of neuromasts (detail) around the mouth. (f) 92 hph: pointed teeth without cusps. arrow, denticles; M, mouth; OC, oral cavity; and YS, yolk sac

the larvae receive their nutrition from the yolk sac, which is constantly being reabsorbed through endocytosis by the yolk syncytial layer (Pena, Dumas, Villalejo-Fuerte, & Ortiz-Galindo, 2003; Shahsavarani, Thomas, Ballantyne, & Wright, 2001). Nakatani et al. (2001) reported that yolk absorption time is a biological parameter that varies among fish species.

During the transition period from endogenous (yolk) to exogenous feeding (live feed), a decrease in the body weight of the larvae was observed at 68 hph, but total length was not affected. This reduction in body weight may be related to the complete absorption of the yolk sac, as well as the depletion of the energy reserves in the liver.

The mouth of *B. splendens* larvae changed disposition during development, acquiring a dorso-terminal position. The position, shape and size of the mouth are strongly correlated with the feeding habit of the fish (Keast & Webb, 1966) and have a quantitative and qualitative influence on the food ingested (Hyatt, 1979). Pointed teeth devoid of cusps are typical of predatory carnivorous species (Al-Hussaini, 1952; Menin & Mimura, 1991; Rodrigues & Menin, 2006), one of the factors that determine *B. splendens* as carnivores.

The oral valves observed in *B. splendens* larvae are similar to those described by Rodrigues and Menin (2006) in *Salminus brasiliensis*. These structures play an important role in respiratory mechanics and

prevent prey from escaping (Rodrigues & Menin, 2006). Taste buds appeared in the buccopharyngeal cavity at 32 hph. The presence of taste buds has also been reported on and in the oral cavity of Tor tambroides at 4 days post-hatching (Ramezani-Fard et al., 2011) and of *Cichlasoma urophthalmus* at 3 dph (Cuenca-Soria et al., 2013). This tasting ability in the buccopharyngeal cavity enables the fish to decide whether to swallow or reject a food item that has been apprehended (Kasumyan, 1999).

The fish oesophagus is a tubular organ that enables the passage of the food bolus from the buccopharyngeal cavity to the stomach. The oesophagus of *B. splendens* appeared as a short, pleated tube; the same pattern of internal oesophageal mucosa folds, arranged longitudinally and in parallel, was described in *Steindachnerina notonota* (Silva, Gurgel, & Santana, 2005) and in *Leporinus macrocephalus* (Rodrigues, Navarro, & Menin, 2008). This pattern of oesophageal mucosa might be related to the ability of the oesophageal wall to distend, which makes it suitable for receiving and rapidly conducting whole prey into the stomach (Amaral, 1990; Diaz, Garcia, Figueroa, & Goldemberg, 2008). In physostomous species, the pneumatic duct of the swim bladder opens into the oesophagus (Zavala-Camin, 1996), a feature also observed in *B. splendens*.

In the present study, lipid accumulation was observed in the liver of the larvae during yolk depletion. It is assumed that before this stage, energy intake is preferentially directed to organ development due to the limited differentiation of the digestive system. Prior to yolk depletion, the energy was accumulated and directed to the liver in preparation for the feeding transition. The use of the liver as an indicator of the nutritional condition in fish is well known. Different studies have shown that structural alterations of the liver due to nutritional imbalances can provide useful information on the quality of the diet and metabolism, complementing the information obtained from growth studies to evaluate the nutritional status of the fish (Gisbert, Villeneuve, Zambonino-Infante, Quazuguel, & Cahu, 2005).

Goblet cells were found in the pharynx and oesophagus of *B. splendens* larvae. According to Sarasquete, Gisbert, Ribeiro, Vieira, and Dinis (2001), these cells are involved in the lubrication of mucosa and in the protection against physical and/or chemical harm caused by the ingestion of prey, and even against bacterial infection. Similarly to most studies, the present work classified mucus-secreting cells as goblet or mucosecretory cells irrespective of their location, as described by Cuenca-Soria et al. (2013). Nevertheless, some studies have differentiated these two types of cells: the former being found in the oesophagus and with a saccular shape, the latter in the intestine, as described by Leknes (2011) for *Hyphessobrycon anisitsi*.

The ontogeny of the digestive tract of *B. splendens* occurred simultaneously with the differentiation of structures that enable escape from predators and the apprehension of food before the depletion of the endogenous energy reserves, such as sensory structures (eyes and neuromasts), locomotion (fins) and the swim bladder, thus increasing the chances of larval survival. According to Paes, Makino, Vasquez, Kochenborger, and Nakaghi (2011), the development of morphological structures that enables the capture of food even before the depletion of endogenous energy reserves increases the chance of survival during the larval stage.

This study demonstrates that the digestive tract of *B. splendens* larvae, reared at 28.0 ± 0.2°C, was fully developed by 74 hph. Therefore, it is recommended that live feed (*Artemia* sp.) only be provided from 74 hph onwards, as by then the larvae would be able to ingest and digest the food. Furthermore, the information from this study on the ontogeny and histophysiology of the digestive tract of Siamese fighting fish can be used to improve larval survival rates and husbandry techniques, and aid in the formulation of diets better suited for ornamental fish larvae.

ACKNOWLEDGEMENTS

This research was approved by the Ethics Committee on the Use of Animals of the Univ Estadual Paulista – UNESP (CEUA, protocol 018092/111), in accordance with the ethical principles promoted by the Brazilian College of Animal Experimentation (COBEA). The authors would like to thank Mr Valdecir Fernandes de Lima from the Laboratory of Ornamental Fish of UNESP's Aquaculture Centre (CAUNESP); Mr Orandi Mateus; Mr Breno Manzini; Ms Sherryl Johana Corchuelo Chavarro; Dr Jõao Ademir de Oliveira; Mr Christian H. C. Flaker; Ms Cláudia Aparecida Rodrigues and Mrs Cláudia R. S. Ribeirinho for technical assistance. This research was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under process no. 131977/2011-3. The authors declare that they have no conflict of interest.

REFERENCES

Al-Hussaini, A. H. (1952). The feeding habits and guts of teleosts, especially of the Northern Red Sea. *Revue de la Faculté des Sciences de l'Université d'Istanbul B*, *17B*, 121–129.

Amaral, A. A. (1990). Anatomia comparativa do aparelho digestório de *Acestrorhynchus britskii* Menezes, 1969 e *Acestrorhynchus lacustris* Reinhardt, 1874 (Pisces, Characidae, Acestrorhynchidae). *Revista Ceres*, *37*, 277–288.

Bértin, L. (1958). Appareil digestif. In P. P. Grassé (Ed.). Traité de zoologie, anatomie, systématique, biologie, vol.*13*, (pp.1249–1301. Paris: Masson.

Chapman, F. A., Fitz-Coy, S. A., Thunberg, E. M., & Adams, C. M. (1997). United States of America trade in ornamental fish. *Journal of the World Aquaculture Society*, *28*, 1–10.

Cuenca-Soria, C., Álvarez-González, C., Ortiz-Galindo, J., Tovar-Ramírez, D., Guerrero-Zárate, R., Aguilar-Hernández, S. … Gisbert, E. (2013). Histological development of the digestive system of Mayan cichlid *Cichlasoma urophthalmus* (Günther 1862). *Journal of Applied Ichthyology*, *29*, 1304–1312.

Diaz, A. O., Garcia, A. M., Figueroa, D. E., & Goldemberg, A. L. (2008). The mucosa of the digestive tract in *Micropogonias furnieri*: A light and electron microscope approach. *Anatomia Histologia and Embryologia*, *37*, 251–256.

Giannecchini, L. G., Massago, H., & Fernandes, J. B. K. (2012). Effects of photoperiod on reproduction of Siamese fighting fish *Betta splendens*. *Revista Brasileira de Zootecnia*, *41*, 821–826.

Gisbert, E., Villeneuve, L., Zambonino-Infante, J., Quazuguel, P., & Cahu, C. (2005). Dietary phospholipids are more efficient than neutral lipids for long-chain polyunsaturated fatty acid supply in European sea bass *Dicentrarchus labrax* larval development. *Lipids*, *40*, 609–618.

He, T., Xiao, Z., Liu, Q., Ma, D., Xu, S., Xiao, Y., & Li, J. (2012). Ontogeny of the digestive tract and enzymes in rock bream *Oplegnathus fasciatus*

(Temminck et Schlegel 1844) larvae. *Fish Physiology and Biochemistry*, *38*, 297–308.

Hyatt, K. D. (1979). Feeding strategy. In W. S. Hoar, D. J. Randall & J. Brett (Eds.), *Fish physiology* (pp. 71–119). New York, NY: Academic Press.

Kasumyan, A. O. (1999). Olfaction and taste senses in sturgeon behaviour. *Journal of Applied Ichthyology*, *15*, 228–232.

Keast, A., & Webb, D. (1966). Mouth and body form relative to feeding ecology in the fish fauna of a small lake, Lake Opinicon, Ontario. *Journal of the Fisheries Board of Canada*, *23*, 1845–1874.

Kjørsvik, E., Pittman, K., & Pavlov, D. (2004). From fertilisation to the end of metamorphosis - functional development. In E. Moksness E. Kjørsvik & Y. Olsen (Eds.), *Culture of cold-water marine fish* (pp. 204–278). Carlton, Victoria: Blackwell Publishing.

Kolkovski, S. (2001). Digestive enzymes in fish larvae and juveniles—implications and applications to formulated diets. *Aquaculture*, *200*, 181–201.

Leknes, I. L. (2011). Histochemical studies on mucin-rich cells in the digestive tract of a teleost, the Buenos Aires tetra (*Hyphessobrycon anisitsi*). *Acta Histochemica*, *113*, 353–357.

Maciel, C. M. R. R., Maciel, A. Jr, Lanna, E. A. T., & Menin, E. (2009). Anatomia funcional da cavidade bucofaringeana de trairão, *Hoplias lacerdae* Miranda Ribeiro, 1908 (Characiformes, Erythrinidae). *Biotemas*, *3*, 95–102.

Menin, E., & Mimura, O. M. (1991). Anatomia funcional da cavidade bucofaringeana de *Hoplias malabaricus* (Bloch, 1974) (Characiformes, Erythrinidae). *Revista Ceres*, *38*, 240–255.

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

Nakatani, K., Agostinho, A. A., Baumgartner, G., Bialetzki, A., Sanches, P. V., & Cavicchioli, M. (2001). *Ovos e larvas de Peixes de Água Doce: Desenvolvimento e Manual de identificação*. Eduem/Nupélia: Maringá.

Paes, M. C. F., Makino, L. C., Vasquez, L. A., Kochenborger, J. B. F., & Nakaghi, L. S. O. (2011). Early development of *Astronotus ocellatus* under stereomicroscopy and scanning electron microscopy. *Zygote*, *20*, 269–276.

Papandroulakis, N., Mylonas, C., Maingot, E., & Divanach, P. (2005). First results of greater amberjack (*Seriola dumerili*) larval rearing in mesocosm. *Aquaculture*, *250*, 155–161.

Pena, R., Dumas, S., Villalejo-Fuerte, M., & Ortiz-Galindo, J. L. (2003). Ontogenetic development of the digestive tract in reared spotted sand bass *Paralabrax maculatofasciatus* larvae. *Aquaculture*, *219*, 633–644.

Ramezani-Fard, E., Kamarudin, M., Harmin, S. A., Saad, C. R., Abd Satar, M., & Daud, S. K. (2011). Ontogenic development of the mouth and digestive tract in larval Malaysian mahseer, Tor tambroides Bleeker. *Journal of Applied Ichthyology*, *27*, 920–927.

Rodrigues, S. S., & Menin, E. (2006). Anatomia da cavidade bucofaringeana de *Salminus brasiliensis* (Cuvier, 1817) (Pisces, Characidae, Salmininae). *Biotemas*, *19*, 41–50.

Rodrigues, S. S., Navarro, R. D., & Menin, E. (2008). Anatomia do tubo digestório de *Leporinus macrocephalus* Garavello & Britski, 1988 (Characiformes, Anostomidae) em relação ao seu hábito alimentar. *Bioscience Journal*, *24*, 86–95.

Sarasquete, C., Gisbert, E., Ribeiro, L., Vieira, L., & Dinis, M. (2001). Glyconjugates in epidermal, branchial and digestive mucous cells and gastric glands of gilthead sea bream, *Sparus aurata*, *Senegal sole*, *Solea senegalensis* and *Siberian sturgeon*, *Acipenser baeri* development. *European Journal of Histochemistry*, *45*, 267.

Shahsavarani, A., Thomas, Z., Ballantyne, J., & Wright, P. (2001). A novel technique for the separation of yolk from the developing embryonic tissue in a teleost fish, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry*, *24*, 321–326.

Silva, N. B., Gurgel, H. C. B., & Santana, M. D. (2005). Histologia do sistema digestório de saguiru *Steindachnerina Notonota* (Miranda Ribeiro, 1937) (Pisces, Curimatidae) do Rio Ceará Mirim, Rio Grande do Norte, Brasil. *Boletim do Instituto de Pesca*, *31*, 1–8.

Støttrup, J., & Norsker, N. (1997). Production and use of copepods in marine fish larviculture. *Aquaculture*, *155*, 231–247.

Tolosa, E. M. C., Rodrigues, C. J., Behmer, O. A., & Freitas Neto, A. G. (2003). *Manual de técnicas para histologia normal e patológico*. Barueri: Manole.

Wootton, R. J. (1990). *Ecology of teleost fishes*. London: Chapman & Hall Publishing.

Yúfera, M., & Darias, M. (2007). The onset of exogenous feeding in marine fish larvae. *Aquaculture*, *268*, 53–63.

Zavala-Camin, L. A. (1996). *Introdução ao estudo sobre alimentação natural de peixes*. Maringá: Eduem.

Zuanon, J. A. S., Salaro, A. L., Veras, G. C., Tavares, M. M. T., & Chaves, W. (2009). Tolerância aguda e crônica de adultos de beta, *Betta splendens*, à salinidade da água. *Revista Brasileira de Zootecnia*, *38*, 2106–2110.