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LARVAL ORGANOGENESIS IN GOLD GOURAMI (TRICHOPODUS TRICHOPTERUS PALLAS 1770) HISTOLOGICAL OBSERVATIONS

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ABSTRACT

This study informs on the ontogeny of larval development of gold gourami, commercially precious fresh-water aquarium fish. Starting from the newly hatched larvae until the days 36-40 when larval development was completed, the main histological findings of the early stages of the development of gold gourami were presented. Gold gourami larvae were altricial at the hatching time, with an undifferentiated digestive tract and a large yolk sac, which was completely consumed within 7-8 days after hatching (DAH). The mouth opened 3DAH and the digestive tract was differentiated with distinct oesophagus, stomach, midgut and hindgut at 4-5DAH. On the 11-13th days, labyrinth organ specific to the fish species with labyrinth was observed to be formed. It was also found that the digestive tract was completely formed, and the number of intestinal folds increased. On the 15-19th days, swim bladder was seen to grow in total without leaving any lobe. It was observed that the brain was divided into telencephalon, diencephalon, mesencephalon and rhombencephalon parts, respectively. Day days, the formation of the gill lamella and caudal fin development were completed. On the 36-40th days, the larval period ended with the formation of the fin rays. Larval development was completed and reached juvenile stage.

KEYWORDS:
Labyrinth fish, anabantidae, gourami, larval development, ornamental fish

INTRODUCTION

All around the world, the popularity of fish keeping has increased by 14% each year since the 1970s and now, over 1 billion individual fish are sold internationally every year [1]. The global ornamental fish trade (both imports and exports) has risen consistently since 2007, and reached from US $ 362 million to a peak of US $ 592 million in 2016 [2]. The industry of the ornamental fish trade is worth of multibillion-dollars in more than 125 countries. Over 2500 species of fish are included, over 60% of which are freshwater origins [3]. More than half (> 90%) of freshwater ornamental fish are captive bred. One of the freshwater ornamental fish, three-spot gourami (Trichopodus trichopterus Pallas 1770; formerly known as, Trichogaster trichopterus) is from the family of Anabantidae, which includes three families, 19 genera and about 120 species in most of southern Asia, India and Central Africa [4]. This species is multi-spawning and male-dependent, which has asynchrony ovary development [5]. Similar to the most fish belonging to this family, the three-spot gourami builds bubble nest and in addition to the fry, their eggs are lighter than water and float on the top of the water [6, 7]. This species can be said to be a popular aquarium fish. Its import to Europe alive dates back to 1896. Since it is easy to keep and breed, the diversity of colours has developed. There are three most popular colour variants; "Cosby", "Golden or Gold" and "Silver". The '"Cosby"' type of gourami has dark blue mottled patches on a light blue body, but the intense gold and silver colors are dominant in the "golden" and "silver" ones, which are tinged with a green or sometimes red hue, respectively [8].

The present study investigated histological events throughout the development of gold gourami larvae (1–29 days after hatching). Trichopodus trichopterus is a very important ornamental species for fish farming. However, although it is very important commercially, there are not so much information about its ontogeny and its larval developmental stages [9]. Therefore, it is necessary to study the larval developmental stages in order to identify well-defined production methods. The early life stages of the three-spot gourami T. trichopterus was morphologically defined by [9]. However, the studies where the larval developmental stages of this species are characterized by histological findings are limited. In addition to the morphological examination of the larval development stage of a commercially important species, the investigation by histological findings is very important to obtain more detailed information about that species.

Histological findings show structural differences during the development of larvae, which provides important information about gourami biology. Early larval developmental stage is a critical stage of

life, starting after hatching, when yolk sac is consumed, the mouth is opened, and the development of the digestive system and other organs is completed [10]. The mortality rates are high in this stage when the digestive glands, enzyme activity and organ development are not yet complete [11]. Therefore, the larval development stage of the species should be well understood. In this study, the larval development stage of T. trichopterus (1-29 days after hatching) was examined histologically. New information on early life stages can enable the development of the current methods for production and cultivation of commercially precious ornamental fish species such as T. trichopterus. Therefore, the histological findings related to the early development about T. trichopterus are thought to be important for many similar species.

MATERIALS AND METHODS

Spawning, hatching and rearing larvae. The spawners used to obtain larvae were obtained from Çanakkale Onsekiz Mart University Aquarium Fish Production and Breeding Laboratory. Production and larval rearing were also carried out in this laboratory. A healthy male and a female spawner that are 1 year old were selected from spawner tanks where there was tens of gold gourami for production. These spawners were taken into the previously prepared production tank. As a production tank, a small glass aquarium in the size of 40 cm x 30 cm x 35 cm (length x width x height) divided by a glass piece was used. The production tank was filled with about 24-25 L of rested water at a height of 20 cm (pH=7). The water temperature was kept stable at 28°C during the trial. The water temperature was adjusted to the desired degree by heating the laboratory environment. There were no filter and no ventilation in the production tank. One female with bulging stomach and one male with a good morphological appearance, chosen from the mixed spawner tank, were separately stocked in two compartments. The glass partition in the middle of the production tank prevented the contact of the male and female contact. The male began to make foam nest within a day or two after seeing the female. Small styrofoam pieces floating above the water were used to prevent the foam nest from scattering. The glass partition between the male and the female was removed when the man made enough foam. After removing the glass piece, the male brought the female to the bottom of the foam nest and started chasing her to lay eggs. On the same day, the male wrapped the female's body with his own body and squeezed her in a way that her abdomen was upwards and then, the ovulation was realized. Ovulation took several hours. During this reproductive behaviour, while the male squeezed the female's body and made her lay eggs, he released the sperms and fertilized the eggs. A few hours after the end of ovulation, the female was moved away from the spawner production tank. The male was left in the production tank with the eggs. Like all other foam nesting species, the male of the golden gourami also cared the eggs. The male kept all the white eggs in the foam nest on the water surface. The eggs were opened at approximately 48-50 hours after 2 days. After the eggs were opened, the larvae were kept together with their fathers in the production tank for approximately 1 week until they could start to swim free and receive feed. Parental care continued during this period. One week after the opening of the eggs, male was removed from the spawner production tank and the larvae were left alone. Larvae samples were taken as of the first day after hatching. After hatching, larvae egg yolk was started to be given from the second day. The diluted egg yolk was given with a 10 mL straw two times a day in the morning and evening. On the 2nd and 5th days, egg yolk was given to feed the larvae while Artemia nauplii (± 430 µm; INVE Aquaculture Inc., Belgium) was started to be given as of 3. day. So, the larvae were fed by both egg yolk and Artemia nauplii (5–10 nauplii mL-1) on the 3rd and 5th days. From the 5th day until the 40th day, the end of the experiment, they were fed only by Artemia nauplii. No water changes were made on the production tank until the seventh day after the larvae emerged. After the 7th day, until the end of the trial (40 DAH) daily 10% water change was made.

Histological observations. For histological evaluations, only healthy and feeding larvae were chosen. The larvae were randomly collected (n = 10) on a daily basis from hatching until the end of the experiment at 40 DAH. The larvae were fixed in Bouin's solution and 70% alcohol, dehydrated by a series of alcohol concentrations, cleared in xylene and embedded in paraffin wax. Wax blocks were cut using a microtome (Slee, Cut5062, Germany) at 5µm. Sagittal sections were stained with Gill's haematoxylin / eosin (HE) procedures for general histology. Sections were observed under a light microscope (Olympus BX50) to describe the larval development and photographed using a colour video camera.

RESULTS

Day 1 (early): The neural tube and notochord are observed in the median line of the larva (Figure 1). There are neural crest cells migrating at the sides of the neural tube. The somites have begun to take shape close to the middle of the larva's body (Figure 1). At the bottom of the notochord, there is the archenteron. There are pronephric tubules in lateral of the archenteron. Yolk sac is very large and filled with pigmented vitellus (Figure 1).

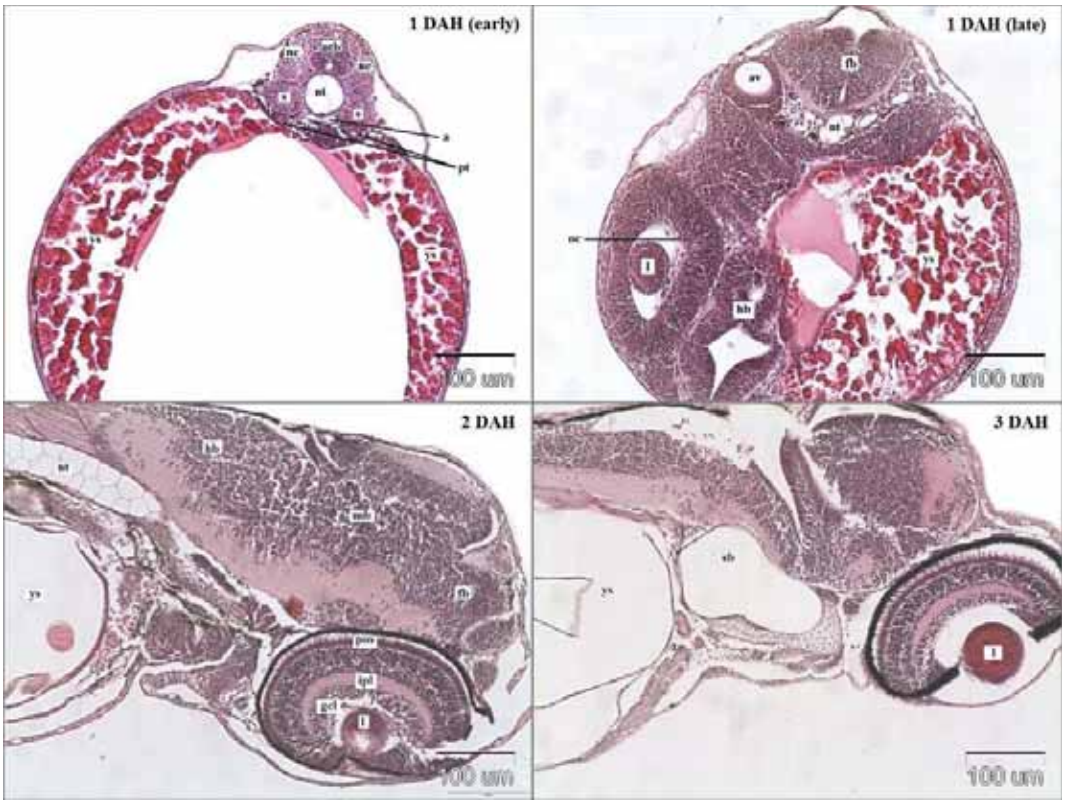

FIGURE 1
Sagittal sections of gold gourami larvae. 1 DAH (early), 1 DAH (late), 2 DAH and 3 DAH.

Day 1 (late): The vitellus material in larvae exists and takes a large space (Figure 1). It is observed that the neural tube is divided into 2 parts as a forebrain and hindbrain (Figure 1). Along with the development of optical capsule and lens, the hearing capsule continues its development on the lateral side of the brain (Figure 1). The lens is surrounded by single-layer cylindrical cells. Mesenchymal cells are observed in the area outside the brain ventricles. Blood vessels are developing.

Day 2: Three parts of the brain are distinguished (forebrain, midbrain and hindbrain) (Figure 1). The presence of notochord is observed from hindbrain towards posterior. There is an increase in the number of somites (Figure 1). In the earlier stage, the optical capsule is observed to be formed since optical vesicles formed in the lateral part of the forebrain become multifold. There is pigmentation within the lens (Figure 1). It is observed that the photoreceptor cells composed of specialized neurons in the eye form a single-row layer. Erythrocytes are seen at the anterior of the yolk sac. The gill organization is just beginning (Figure 1). There is a reduction in the amount of vitellus in the yolk sac. The caudal region can be distinguished.

Day 3-4: It is observed that the swim bladder has developed in the posterior part of the eye (Figure 1). Yolk sac is narrowed on the posterior part, but it takes a wide place in the anterior part. The pigmentation within the lens can be seen clearly. Gill springs (4 pieces) are observed (Figure 1). The mouth is fully opened, the teeth are observed at the end of the mouth opening. The digestive tract continues to develop, and pharynx is seen in the posterior of the mouth opening.

Day 5-7: It is observed that the digestive tube is formed (Figure 2). It is possible to differentiate between the stomach and intestine thanks to the epithelial layers. It is possible to conclude that the larva is fed from the external source of nutrients thanks to the pieces of food in the stomach and intestine folds. The yolk sac is tightly attached to the anterior (Figure 2). An endoderm-originated liver pattern is observed. It is possible to see pronephric tubules in the cavity where the kidney will be formed (Figure 2). The number of somite has increased. Head, body and tail can be distinguished.

Day 11-13: It is observed that gill lamellae develop (Figure 2). There is a pouch-shaped heart in the posterior part of the gill springs. Erythrocytes in the heart are quite obvious. In the ventral of the heart, there is a labyrinthine organ that is unique to this species. Cells and sinusoids are evident in the liver (Figure 2). It is seen that the digestive tract is completely formed, and the number of intestinal folds increases.

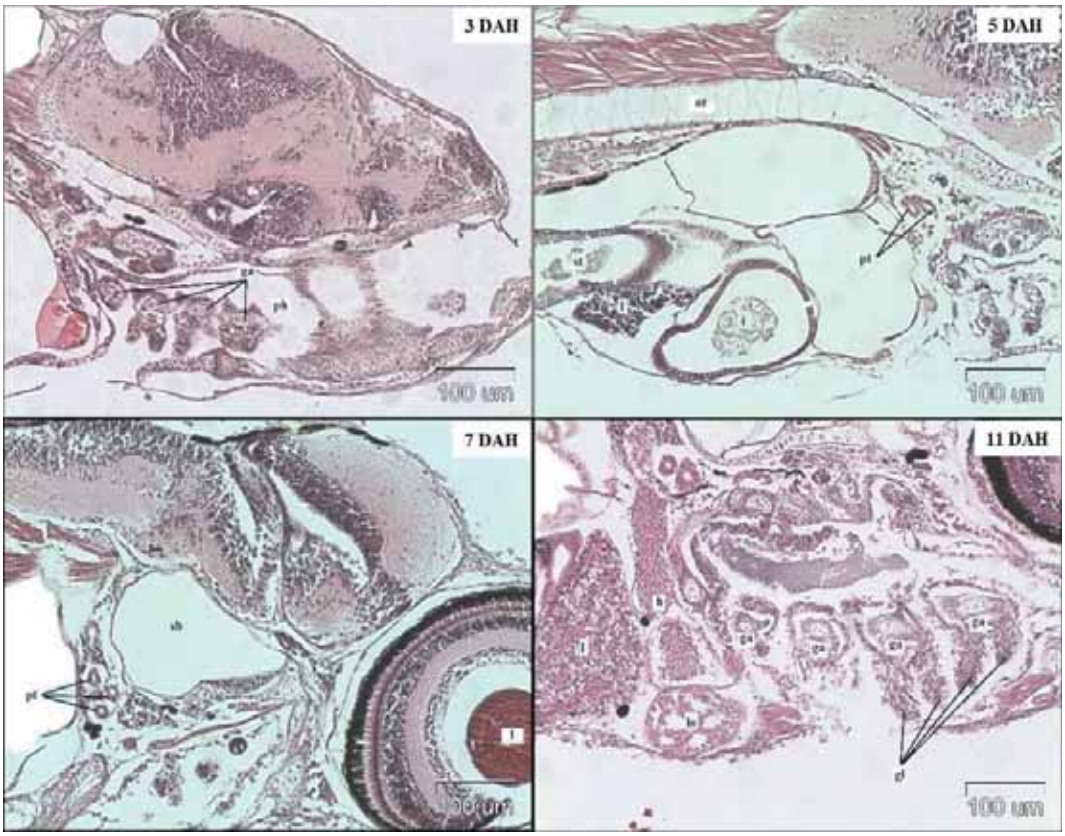

FIGURE 2
Sagittal sections of gold gourami larvae. 3 DAH, 5 DAH, 7 DAH and 11 DAH.

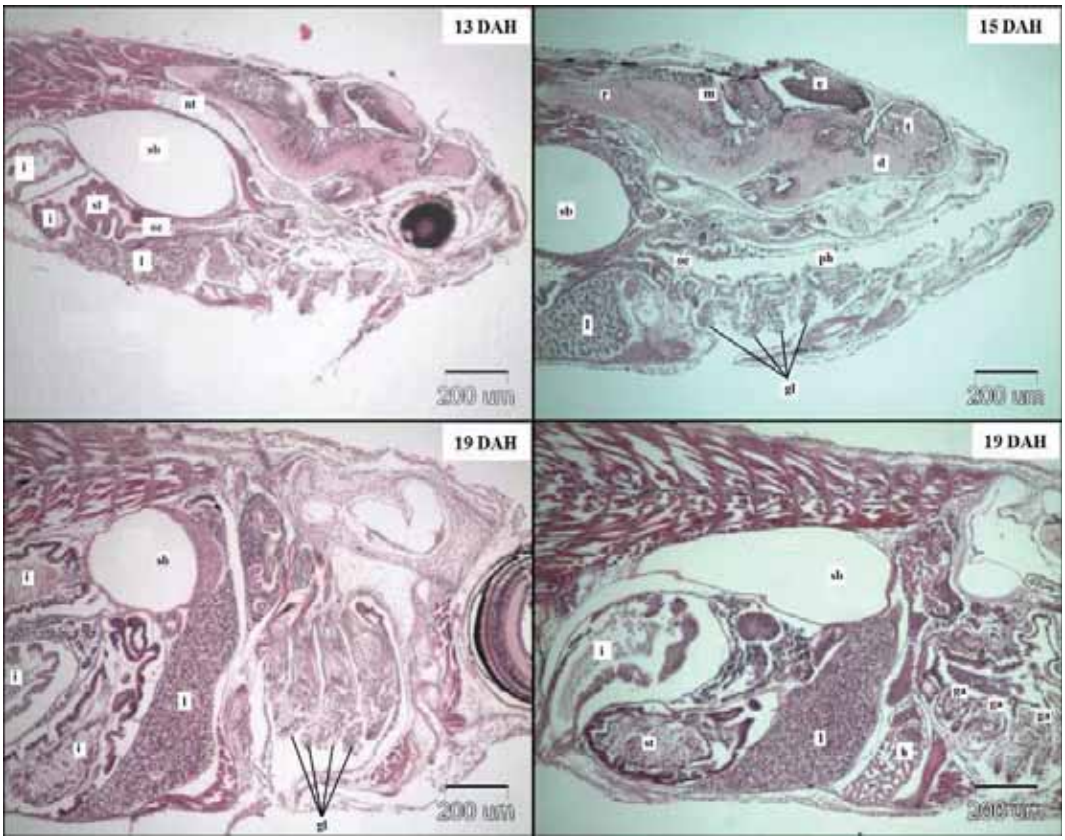

FIGURE 3
Sagittal sections of gold gourami larvae. 13 DAH, 15 DAH, 19 DAH and 19 DAH.

Day 15-19: It is observed that the swim bladder grows completely without leaving any lobe (Figure 3). The anterior part of the swim bladder covers a larger volume than the posterior part. Brain parts can be seen obviously (Figure 3). They are respectively telencephalon, diencephalon, mesencephalon and rhombencephalon. In addition, epiphysis formation is observed (Figure 3).

Day 21-25: Larvae continue to increase in length. It was identified that the gill organization was completely formed and gill lamellae were clearly observed. It is seen that the caudal fin completed its development. The cartilage elements of the skull skeleton are obvious.

Day 27-32: Since new findings showing development have not been found, it can be said that the larval development phase has started to be completed nowadays. The morphological appearance of the larvae is much more similar to the body of their parents.

Day 36-40: Particularly on the 40th day, the larval period was completely terminated. All of the structures are similar to the full-grown and especially fin rays are clearly seen.

Our results indicate that the ontogeny of larval development of gold gourami followed the general pattern reported in the current literature for most fish species, such as Brycon gouldingi (Teleostei, Characidae) [12], Trichogaster pectoralis (The snakeskin gourami, Osphronemidae) [13], Osphronemus goramy (The giant gourami, Perciformes: Osphronemidae) [14], Symphysodon spp. (Discus, Cichlidae) [15], Pterophyllum scalare (The freshwater angelfish, Teleostei: Cichlidae) [16], Brycon amazonicus (Teleostei, Bryconidae) [17], Betta splendes (The Siamese fighting fish, Anabantoidei) [18], and Gymnocorymbus ternetzi (The black skirt tetra, Characidae) [19], This information can be helpful in enhancing the success of rearing the most of freshwater ornamental fish larvae.

In most fish species, the newly hatched larvae have undifferentiated digestive tracts [11]. Gold gourami larvae have a simple undifferentiated digestive tract, as in many other fish species [20-23, 12]. Similarly, while gold gourami larvae have a rudimentary digestive tract at the time of hatching, they have the digestive tract divided into gut head, foregut, midgut and hindgut at the time of total yolk absorption. The same classification was adopted by [24]. The basic mechanisms of organ and digestive system development are similar in all teleosts, but the timing of development, differentiation and functioning of organs is different during early ontogeny [25]. A differentiated pancreas and liver are usually functional before the yolk is completely consumed [12], which is confirmed in Trichogaster pectoralis [13], Osphronemus goramy [14], Betta splendes [18], Symphysodon spp. [15], Pterophyllum scalare [16], Brycon amazonicus [17] and Gymnocorymbus ternetzi [19], also found in the present study.

In salmonids and some cichlid species, newly hatched larvae have pigmented eyes, their lower jaws are formed and fin rays are visible [26]. The newly hatched gold gourami larvae do not have pigmented eyes, their lower jaws are not formed and their fin rays are not developed. This is similar in many species of freshwater aquarium fish [13, 14, 16, 18, 19]

In many fish species, newly hatched larvae have cement glands over the head, such as all the mouth-brooding species [27], Symphysodon spp. [28], Symphysodon aequifasciatus [29], Pterophyllum scalare [16] and Gymnocorymbus ternetzi [19]. In contrast, gold gourami larvae do not have a cement gland during early developmental stages.

Our results indicated that mouth opening in larval gold gourami occurs within 3 DAH. However, [9] reported that mouth opening in three-spot gourami, Trichogaster trichopterus larvae occurs at 1 DAH. In contrast, [30] reported that mouth opening in the dwarf gourami Trichogaster lalius larvae occurred at 3 DAH. This may be due to the fact that the conditions of rearing or observation are different. In larval breeding of commercial fish species known as labyrinthine fish and making foam nests such as gourami and betta, it is important to determine the labyrinth organ development that is specific to these species. Because the labyrinth organ development process is one of the periods with the highest mortality in the larval stage. This period is a very sensitive period for larvae. Therefore, it is very important to know when the labyrinthine organ is formed. In a study conducted by [9], the formation of labyrinth organ in larvae of three-spot gourami, Trichogaster trichopterus was reported to be formed on the 18-20th days. For the giant gourami Osphronemus goramy, it was reported that the labyrinth organ began to develop on day 30 and air breathing behavior was observed on the days 35-40 [14]. According to [13], the labyrinth organ of snakeskin gourami Trichogaster pectoralis differentiated in postflexion larvae stage (day 22).

According to the histological findings presented in this study; at 28°C water temperature, labyrinth organs of golden gourami larvae were observed to be formed 11-13 days after hatching. After the formation of the labyrinth organ, larvae gain the ability to breathe the atmospheric air. Even if the oxygen level in the water is low, gourami fish can use oxygen in the atmospheric air thanks to their labyrinth organs. Therefore, it is necessary to know the exact time of labyrinth organ development of the gourami larvae.

In rearing of larvae, one of the important physiological events that should be identified is the consumption time of the yolk sac [31]. In parallel with the consumption of the yolk sac, digestive system development and external feeding behaviour also

progress. Therefore, the morphological observation of consumption of yolk sac may give an idea about especially the development of the digestive system. In addition, according to these findings, an external feeding program can be adjusted. So it is important to observe the developmental processes of the yolk sac of commercial fish larvae.

In the present study, histological observations indicated that in gold gourami, the yolk sac (vitellus) was completely absorbed within 7-8 days after hatching (DAH). The consumption of the yolk sac was reported to occur on the 11th-15th days after the hatching for some gourami species such as the three-spot gourami, T. trichopterus (11 DAH) [9]; the snakeskin gourami, Trichogaster pectoralis (12 DAH) [13]; the giant gourami, Osphronemus goramy (14-15 DAH) [14]. On the other hand, the consumption of the yolk sac was reported to be on the 3th-4th days after the hatching for some the labyrinth fish species such as the striped gourami Trichogaster fasciatus (2-3 DAH or 50h after hatching) [32], the striped gourami Trichogaster fasciata (3-4 DAH) [33], the Siamese fighting fish Betta splendes (3-4 DAH or 73h after hatching) [18] and the dwarf gourami Trichogaster lalius (4 DAH) [30]. Labyrinth fish have different consumption times of yolk sac. In fact, the same gourami species reared in the same conditions may have different consumption times of yolk sac. Due to many other factors such as water temperature, light and water quality parameters, rearing conditions change. These differences in rearing conditions also affect organ development periods in the larval developmental stage. Therefore, such differences should be taken into account in larval rearing.

In many species of freshwater fish, the larval developmental stage is completed within 30-35 days after hatching and the it reaches juvenile stage [16, 18, 26, 28, 32-36]. Histomorphological findings indicated that the larval developmental stage of gold gourami (T. trichopterus) larvae was completed in 36-40 days after hatching.

CONCLUSIONS

This study presented the histological findings defining the larval period of the golden gourami "Trichopodus trichopterus belonging to the genus, Trichopodus (formerly Trichogaster). There are over one hundred Anabantoids or labyrinth fish species that are currently traded in the global tropical fish industry [37]. The genus Trichogaster (now Trichopodus) contains many of the more popular gouramis traded in the industry, including the "Gold gourami", "Blue gourami", "Three spot gourami", "Silver gourami", "Opaline gourami", "Pearl gourami", "Moonlight gourami", "Snakeskin gourami" and hybrids in the same genus [37].

All of these fish species reproduce and are reared similarly. Their larval developmental stages are similar [37]. Only the time of their organ development is different. Therefore, the findings of this study can be used for all these species belonging to the genus of Trichopodus. It is highly probable that professional producers who rear any type of gourami will benefit from the information presented here. Therefore, gold gourami can be a model for all other labyrinth fish. It is even possible to use these findings in many other types of labyrinth fish. The information presented here is important for both researchers and professional producers as a helpful source.

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