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Morphology and anatomy of larval development in black neon tetra fish *Hyphessobrycon herbertaxelrodi* (Characiformes: Characidae)

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ABSTRACT

Introduction: The black neon tetra (*Hyphessobrycon herbertaxelrodi*) is a popular ornamental fish, and understanding its larval development is crucial for aquaculture practices.
Objective: To examine the developmental stages during the larval period of black neon tetra produced under laboratory conditions.
Methods: Larvae were sampled daily fromhatching until reaching the juvenile stage. Each specimen was photographed and prepared for anatomical observations.
Results: Morphological observations indicated that the mean total length (TL) of the larvae was 2.77 ± 0.08 mm on the first day after hatching (DAH) and increased to 12.26 ± 0.96 mm by the 29th-30th DAH when they reached the juvenile stage. Initially, the fins were in a primordial form, with the mouth and anus opening on the 4th and 6th DAH, respectively. The anal and dorsal fins began to differentiate on the 10th and 14th DAH, coinciding with the completion of notochord flexion on the 12th-13th DAH. The second swim bladder developed on the 14th and 15th DAH, and caudal fin bifurcation was observed on the 18th day. Anatomical observations indicated significant changes in the developmental process of black neon tetra larvae. On the 1 st DAH, the digestive system of the yolk sac larva was in the shape of a flat long tube. By the early 3 rd day, the mouth had opened, and the swim bladder had noticeably swollen, taking the form of a tube in the digestive canal. On the 5 th day, the yolk sac had been depleted, and the stomach and intestine began to develop. By the 10 th day, the liver had replaced the yolk sac, increasing the folds of the stomach and intestine. The juvenile stage commenced on the 29th-30th DAH, marking the end of the larval process.
Conclusions: The findings provide essential insights into the morphological and developmental milestones of black neon tetra larvae, which are vital for enhancing aquaculture techniques and breeding protocols.

Key words: Tetra fish; Characidae; larvae; growth; aquarium.

RESUMEN

Morfología y anatomía del desarrollo larvario del pez tetra neón negro *Hyphessobrycon herbertaxelrodi* (Characiformes: Characidae)

Introducción: El tetra negro (*Hyphessobrycon herbertaxelrodi*) es un pez ornamental popular y entender su desarrollo larval es crucial para las prácticas de acuicultura.
Objetivo: Examinar las etapas de desarrollo durante el período larval del tetra negro producido en condiciones de laboratorio.
Métodos: Se tomaron muestras de larvas diariamente desde la eclosión hasta alcanzar la etapa juvenil. Cada ejemplar fue fotografiado y preparado para observaciones anatómicas.

Resultados: Las observaciones morfológicas indicaron que la longitud total media (LT) de las larvas era de 2.77 ± 0.08 mm el primer día después de la eclosión (DAE) y aumentó a 12.26 ± 0.96 mm para los días 29-30º DAE, cuando alcanzaron la etapa juvenil. Inicialmente, las aletas estaban en una forma primordial, con la boca y el ano abriéndose en el 4to y 6to DAE, respectivamente. Las aletas anal y dorsal comenzaron a diferenciarse en el 10º y 14º DAE, coincidiendo con la finalización de la flexión del notocordio en el 12-13º DAE. La segunda vejiga natatoria se desarrolló en el 14º y 15º DAE, y la bifurcación de la aleta caudal se observó en el día 18. Las observaciones anatómicas indicaron cambios significativos en el proceso de desarrollo de las larvas de tetra negro. En el 1er DAE, el sistema digestivo de la larva del saco vitelino tenía la forma de un tubo largo y plano. A inicios del 3er día, la boca se había abierto y la vejiga natatoria aumentó notablemente, tomando forma de tubo dentro del canal digestivo. En el 5to día, el saco vitelino se había reabsorbido y comenzaron a desarrollarse el estómago y el intestino. Para el 10º día, el hígado reemplazó el saco vitelino, aumentando los pliegues del estómago y el intestino. La etapa juvenil comenzó en los días 29-30º DAE, marcando el final del proceso larval.
Conclusiones: Los hallazgos proporcionan información esencial sobre los hitos morfológicos y desarrollo de las larvas de tetra negro, que son vitales para mejorar las técnicas de acuicultura y los protocolos de cría.

Palabras clave: pez tetra; Characidae; larvas; crecimiento; acuario.

INTRODUCTION

The global aquarium fish trade has transformed into a multi-billion-dollar industry and continues to grow every year. According to report published a few years ago, the global aquarium fish market was valued at USD 4.5 billion in 2020 and is exğected to reach USD 6.3 billion by 2028 (Grand View Research, 2021). Tetra fish, with more than 150 species, are among the important fish groups that are commercially valuable and popular among hobbyists (Çelik & Çelik, 2022). Most living organisms in the business involve fish species of fresh water. The most commercially dominant are the species of Cyprinodontiformes, Perciformes, Characiformes and Siluriformes families (Evers et al., 2019). Black neon tetra *Hyphessobrycon herbertaxelrodi* is the species of ornamental fish included in Characiformes family. The present study morphologically examined stages of larval development in black neon tetra fish produced under laboratory conditions. Knowledge of early stages in a commercial fish species is of great importance for the research conducted in the fields of ichthyology, fisheries biology, protection of ichthyofauna and improvement of aquaculture techniques (Reynalte-Tataje et al., 2020; Rizzo & Godinho, 2003; Santos et al., 2020). Moreover, data on larval development defined by morphological and biometrical parameters are quite essential for fish breeders to develop protocols for production and larva culture. (Lima et al., 2020; Perrotti et al., 2019; Portella et al., 2014). Considering the assessment above, it is assumed that information on early life stages of a commercially precious fish species would be of great use in scientific and commercial terms (Souza da Silva et al., 2022).

Fish development is generally divided into embryo, larva, juvenile, young and adult stages (Urho, 2002). Development of early life stages in fish usually follows the same models until the larval formation (Falk-Peterson, 2005). The larval period is considered one of the most crucial stages in the fish life cycle (Nowosad et al., 2021). The rapid morphological changes and organ development that occur during this phase significantly influence the subsequent development of fish and their survival rates (Song et al., 2019; Zadmajid et al., 2019). Understanding embryology and larval developments in fish has a key role in creating the very approach to their biology and taxonomy (Reynalte-Tataje et al., 2004). Morphological characteristics help obtain comprehensive data of life cycles on one hand and pave the way for valuable traces of human ability to produce and breed fish under aquaculture circumstances on the other (Martinez et al., 2000; Martinez-Lagos & Gracia-Lopez, 2009; Silva, 2004). Data concerning the morphological development of larval stages in fish is crucial for establishing management protocols in aquaculture, especially for creating larviculture protocols (Papadakis et al., 2018; Kupren et al., 2019; Lv et al., 2019; Pepe-Victoriano et al., 2021).

The aim of this study is to examine the developmental stages of black neon tetra (*H. herbertaxelrodi*) larvae produced under laboratory conditions. The black neon tetra is a popular ornamental fish, and understanding larval development is critical for aquaculture practices. In this context, a detailed examination of morphological and histological changes during the larval period will contribute to the development of fish farming techniques and breeding protocols. This study aims to provide the necessary information for the cultivation and conservation of this species by identifying the important stages of the larval development process.

MATERIAL AND METHODS

The study examined the larval development of black neon tetra *H. herbertaxelrodi* used by adult individuals older than one year as broodstocks. *H. herbertaxelrodi* is a tetra species classified in the Characidae family from the Amazon River in origin. The body of *H. herbertaxelrodi* is flat with a slight depth in shape (Fig. 1).

It is silvery grayish black in color. The body has a bright white line from the gill towards the tail along the lateral sites (Fig. 1), which assumes an iridescent white line as if it were a neon light. The species is therefore called "Black Neon Tetra" (Tropical Fish Hobbyist Magazine, 2007). The body of a male is thinner and smaller that of the female in structure.

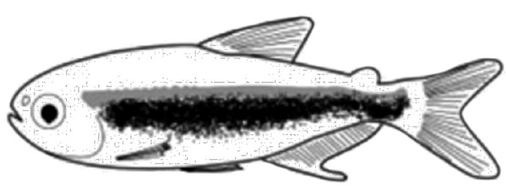

Fig. 1. Morphological appearance of the adult individual of the black neon tetra fish, *Hyphessobrycon herbertaxelrodi* (Original drawing, Çelik, 2011).

All broodstocks were fed three meals a day with the same feed (Protein: 46 %, Oil: 12 % Fiber: 3 %, Ash: 11 %, Moisture: 8 %; Tetramin Granulat, Tetra, Germany). Water quality parameters and spawning tanks were preserved stable in the ranges of 24 ± 0.5 ºC temperature, 6.0-6.5 pH and 100-200 µ in conductivity. 100-watt aquarium heaters were used to keep the water temperature stable. Female and male broodstocks were preserved in 40 l separate glass aquaria (Broodstock aquarium dimensions: length 40 × width 30 × height 35 cm, water depth/height 32-33 cm). The broodstock aquaria were exposed to photoperiod program of 9 h lightness / 15 h darkness. The lights were kept on from 07.00 until 18:00. 3 female / 3 male broodstocks were randomly selected from the aquaria and brought into the 15 l glass tanks for production where broodstocks were seen to spawn by dawn the following day. The eggs gathered around the bottom during the spawning were left there after which the broodstocks were removed from the tanks. Preserved at stable temperature within the range of 24 ± 0.5 ºC, the eggs were left to incubate in the tank for production to follow larval development.

Larval samples were randomly taken every day from the first day of the hatching until their juvenile. Larvae were observed by means of SZX7: Olympus™ zoom stereomicroscope (Tokyo, Japan), were photographed by the attached video camera (Q Imaging, Micropublisher 3.3 RTV, Canada) and were morphometrically measured by the picture analysis program (Q Capture Pro, version 5.1.1.14, Canada). The growth data of the larvae were obtained by measuring the lengths of the larvae photographed under the microscope.

Larvae were fed once a day with brine shrimp nauplii '*Artemia salina*' (INVE Aquaculture Inc., Dendermonde, Belgium) by the end of the experiment on 28 DAH. The feeding rate was 5-10 individuals/ml. *Artemia* cysts were incubated in 1.5 l seawater in a liter scale cylindrical-conical plastic container at 25-26 °C and 25-30 ppm salinity. Aeration was maintained by a small pipeline from an aquarium air pump down the bottom of the hatching device. *Artemia* cysts were hatched within 24-26 h under these conditions. The newly hatched nauplii were harvested and added to the larva tanks by means of a pipette.

Larval stages of development were identified according to Kendall et al. (1983) and were grouped into four periods I: Yolk-sac larva from hatching until depletion the yolk sac, II: Preflexion larva from the depletion of the yolk sac by the beginning of flexion of notochord end III: Flexion larva from the start of notochord flexion until its complexion and IV: Post-flexion larva from the completion of the notochord end flexion by the time when larval development was over and juvenile was about to begin. Larvae finished their development to completely reach the forms of adult individuals following stage IV at which younglings were defined as juveniles then the observations of larval development were concluded.

Anatomical observations: For anatomical assessment, larvae were randomly collected daily from hatching (1 day after hatching = DAH) until juvenile stage. The specimens were fixed in a Bouin's solution and 70 % alcohol, dehydrated through a series of alcohol concentrations and were cleared in xylene and paraffin-waxed. Wax blocks were cut using a microtome (Slee, Cut5062, Germany) at 5 µm. Sagittal sections were stained by Gill's hematoxylin / eosin (HE) procedures for general histology. The blocks were observed under a light microscope (BX50: Olympus™) to describe the larval development and were photographed using a color video camera.

RESULTS

Morphological observations, 1 DAH (TL: 2.77 ± 0.08 mm): The mouth and anus were closed, and the digestive system did not differentiate yet. The eyes did not have pigmentation and star-shaped anophores seemed to be dispersed on the yolk sac and body. Primordial fin developed well, and no other fins differentiated yet. The yolk sac was oval measuring about 20 % of the total length. Since pigmentation did not develop, the outlets could be easily seen through the otic capsule where they were present (Fig. 2a).

2 DAH (TL: 3.34 ± 0.04 mm): The mouth was still closed. The digestive system was in the form of a semitransparent and differentiated flat tube with the yolk sac beginning to lessen. Although pigmentation increases in eye and head sections, other parts of the body seem more transparent. Black melanophores are dispersed or irregularly present in and around the head, flanks and back sections as well. The bladder lies near the anus. The pectoral fin bud was present, but anal and dorsal fins have not formed yet (Fig. 2b).

3 DAH - 4 DAH (TL: 3.49 ± 0.07 mm): The swim bladder began to inflate on the 3rd day. The pigmentation was completed in the eyes. On the 3rd day the mouth was still closed. The digestive system assumed an entire tube. The yolk sac was still present (Fig. 2c). On the 4th day, still existed remains of the yolk sac that were not completely depleted. The mouth opened on the 4th day. The swim bladder extended towards the posterior. The swim bladder enlarged posteriorly. The development of the eye was completed. The primordial fin was present, and the other fins did not differentiate. The larva began to feed exogenously on the 4th day (Fig. 2d).

5 DAH - 8 DAH (TL: 3.70 ± 0.17 mm - 4.51 ± 0.28 mm): There was if little yolk sac even on the 5th day. The bladder has been clearly visible just next to the anus. Pigmentation was increasing (Fig. 2e). The yolk sac was completely depleted on the 6th day. The swim bladder continued to extend posteriorly. Pieces of digested food (*Artemia salina*) in the stomach could be seen (Fig. 2f). The swim bladder continued to enlarge in size. On the 7th day, notochord was not still in flexion but flat in shape (Fig. 2g). Pigmentation continued to

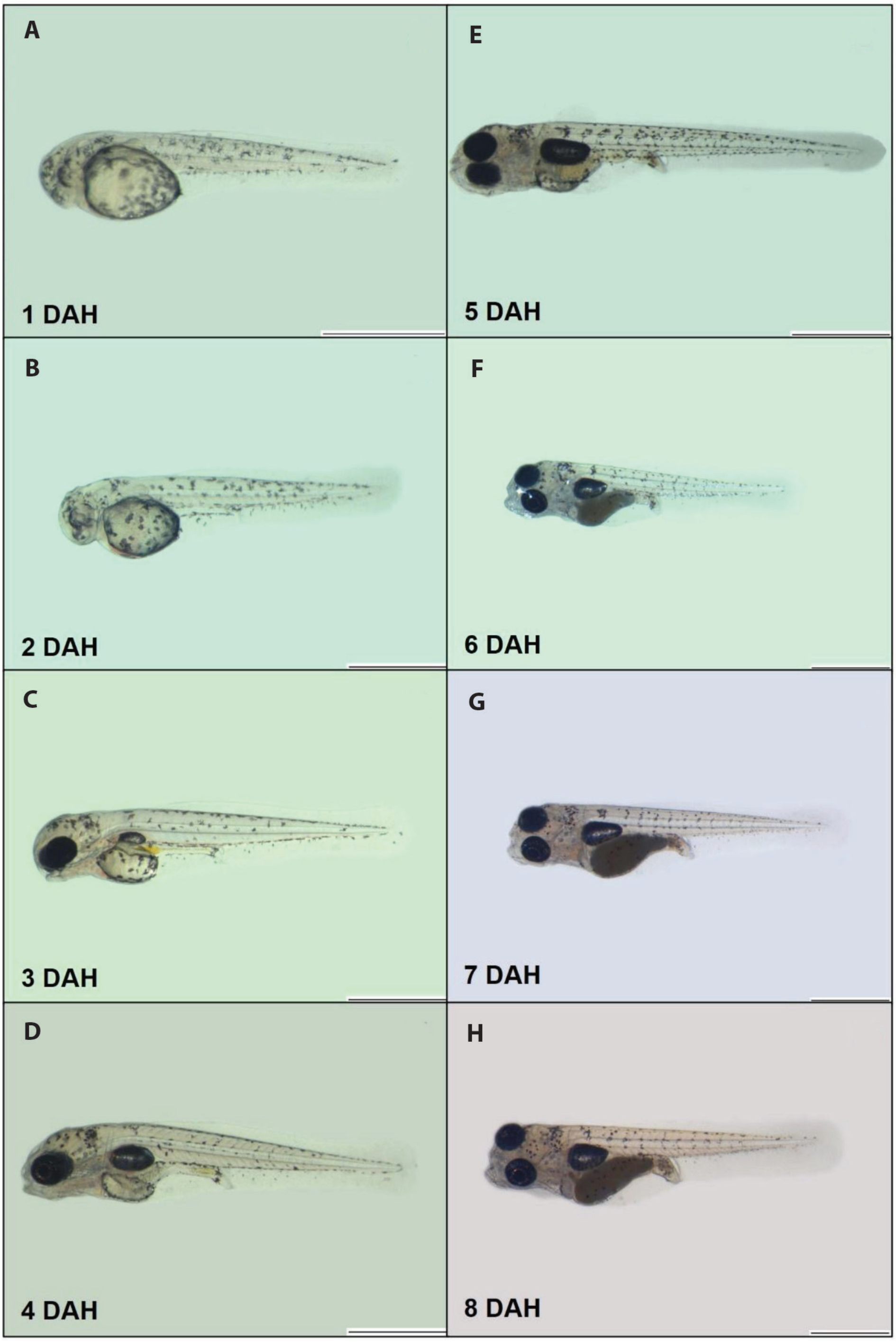

Fig. 2. Larval development stages of black neon tetra *Hyphessobrycon herbertaxelrodi*. 1–8 DAH, 24 ± 0.5 ºC. **A.** Post-hatching yolk sac stage (1 DAH). **B.** Yolk sac stage (2 DAH). **C.** Yolk sac stage, the gas bladder was formed but not completely filled (3 DAH). **D.** Opened-mouth stage (4 DAH). **E.** Preflexion larva, exogenous feeding (5 DAH). **F.-H.** Preflexion larva (6, 7, 8 DAH). Scale bars: 1 mm.

increase. Differentiation of dorsal and anal fins did not occur (Fig. 2h).

9 DAH - 10 DAH (TL: 4.96 ± 0.17 mm - 5.39 ± 0.11 mm): Despite an increase in pigmentation, the body of the larva hardly colored thus appearing semitransparent. The volume of the swim bladder continued to increase (Fig. 3a). The primordial fin that had existed since the early days was present and the anal fin did not differentiate yet. The tail fin rays began to form (Fig. 3b).

11 DAH - 13 DAH (TL: 5.71 ± 0.12 mm - 6.48 ± 0.22 mm): Pigmentation continued to increase all over the body (Fig. 3c). The transparent appearance of the body on early days gradually vanished as the time passed. On the 12th day emerged the flexion on notochord end. The tail fin rays began to become prominent. Differentiation of dorsal and anal fins started (Fig. 3d). Pigmentation increased. The swim bladder enlarged towards the posterior. The tail fin rays increased, thus becoming evident (Fig. 3e).

14 DAH - 16 DAH (TL: 6.61 ± 0.49 mm - 7.68 ± 0.61 mm): On those days, differentiation of the dorsal and anal fins became visible as well. The first swim bladder appeared significantly enlarged and lengthened (Fig. 3f) with the second swim bladder gradually emerging. Dorsal and anal fin rays began to be prominent (Fig. 3g). The number of dorsal and anal fin rays increased to finally become visible to the naked eye. The swim bladders continued to increase in size. Pigmentation went on increasing more than on the previous days (Fig. 3h).

17 DAH - 21 DAH (TL: 7.96 ± 0.35 mm - 9.86 ± 0.42 mm): As the coloration was more intense in head and dorsal areas of the body, they lost their transparent appearance. However, the pigmentation was less in the areas of the abdomen and stomach, thus they inevitably seemed partially translucent such that digested pieces of food (*Artemia salina*) could be seen to remain in the stomach. The dorsal, anal and tail rays became more prominent than they were in previous days. The volume of the swim bladder continued to grow (Fig. 4a). Pigmentation continued to increase the bifurcation on the tail fin was observed to begin (Fig. 4b). There appeared the adipose fin formation between the dorsal and tail fins (Fig. 4c). Pigmentation continued to increase all over the body. Tail bifurcation increased. The dorsal and anal fins continued to develop (Fig. 4d).

23 DAH - 29 DAH (TL: 10.25 ± 0.57 mm - 12.26 ± 0.96 mm): The swim bladder continued to grow, and the pigmentation was increasing. The dorsal, anal and tail fins kept on developing (Fig. 4e). Just beneath the lateral line increased the pigmentation (Fig. 4f). On the 29th-30th days, the larva completed its morphological development (Fig. 4g), following which it reached the juvenile phase. The external appearance of the larva (39 DAH) seemed like in the figure. (Fig. 4h). 10 days after juvenile when body appearances of the younglings morphologically assumed the shapes of mature individuals.

Growth: The growth formula of black neon tetra calculated by the exponential relationship model during the early larval period was as follows; $y = 3.0053e^{0.0531x}$ ($R^2 = 0.9694$, n = 112); where y is the total length (TL) and × DAH. Total length of the larvae was 2.77 ± 0.08 mm, 3.70 ± 0.17 mm, 5.39 ± 0.11 mm, 6.85 ± 0.38 mm, 9.86 ± 0.42 mm and 11.35 ± 0.8 mm on the 1st, 5th, 10th, 15th, 21st and 27th days, respectively. Accordingly, the mean total length of the larva that reached juvenile phase on the 29-30th days was found to be 12.94 ± 1.03 mm (Fig. 5). The size of the yolk sac was measured as 790.26 ± 45.19 µm on the 1st day when it was hatched, followed by 728.39 ± 73.25 µm on the 2nd, 664.52 ± 3rd, 33.15 µm and 562.20 ± 43.40 µm 4th day. The yolk sac measured to be 400.39 ± 227.38 µm after the 5th day was seen to be completely depleted (Fig. 6).

The swim bladder of black neon tetra larva is composed of two sections. The initially formed swim bladder as the main, it begins to

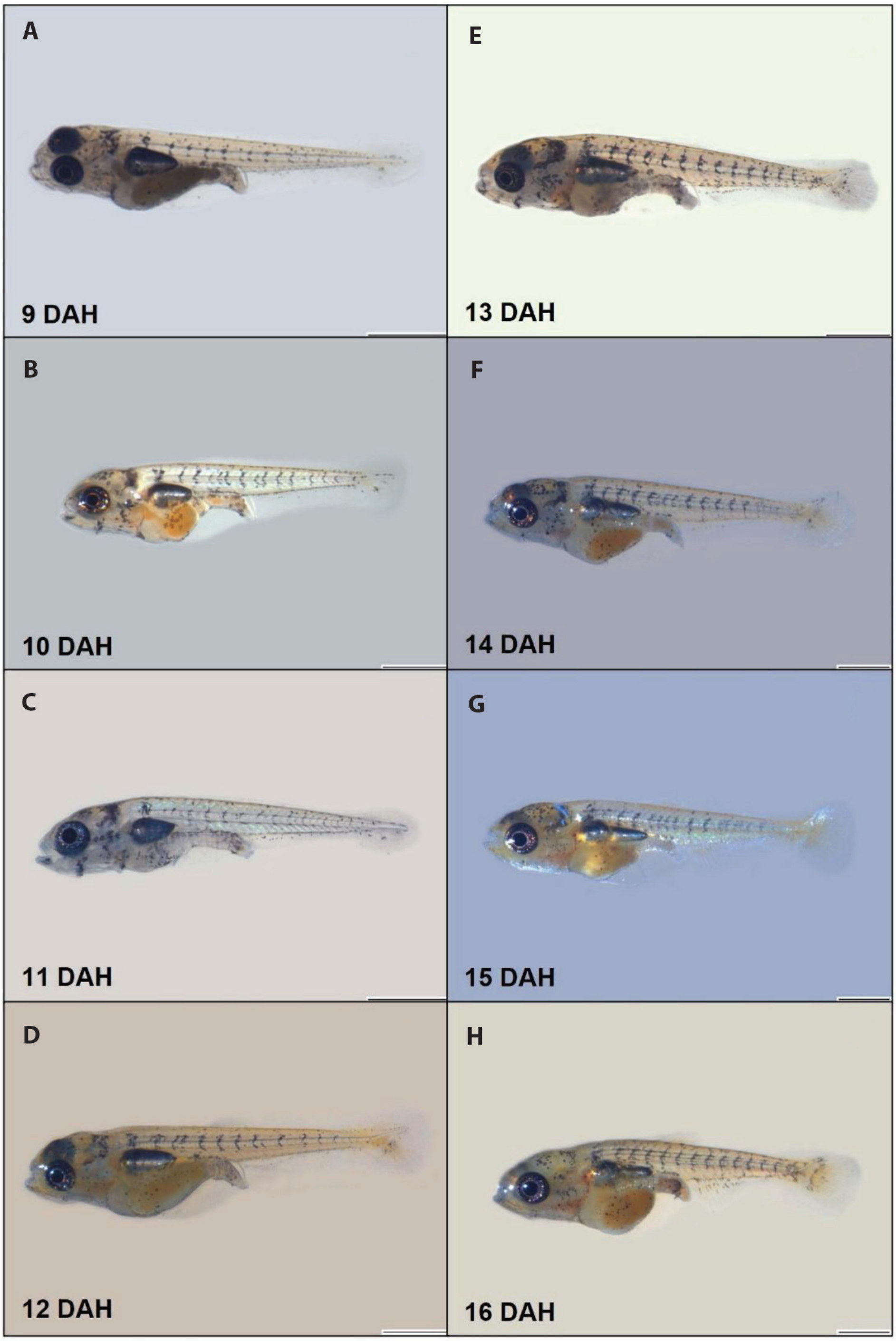

Fig. 3. Larval development stages of black neon tetra *Hyphessobrycon herbertaxelrodi*. 9-16 DAH, 24 ± 0.5 °C. **A.-C.** Preflexion larva (9, 10, 11 DAH). **D.** Flexion stage, notochord flexion started (12 DAH). **E.** Flexion stage, the notochord was completely flexed (13 DAH). **F.-G.** Postflexion larva, second inflation of the swim bladder (14-15 DAH). **F.-H.** Postflexion larva (14, 15, 16 DAH). Scale bars: 1 mm.

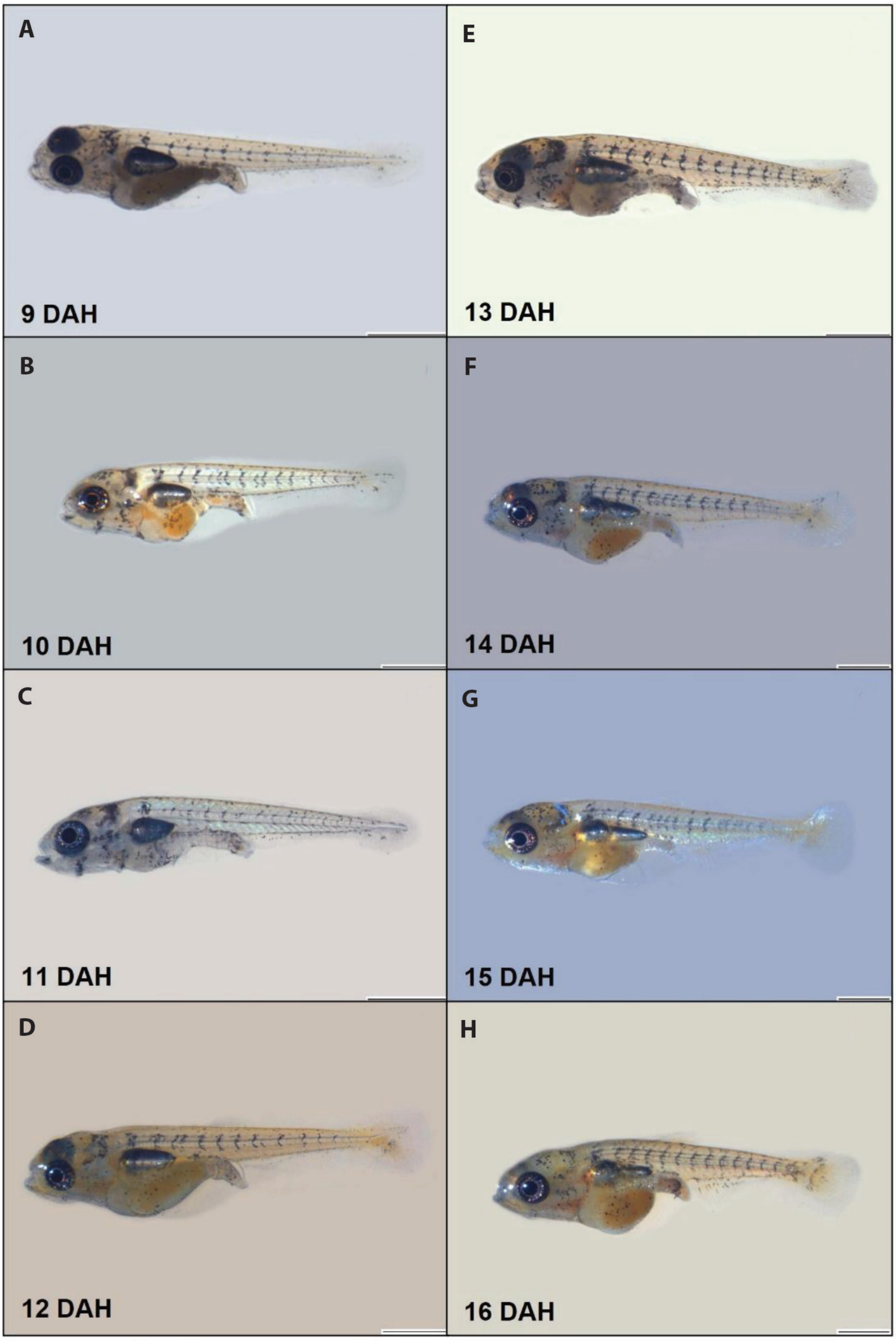

Fig. 4. Larval development stages of black neon tetra *Hyphessobrycon herbertaxelrodi*. 17-39 DAH, 24 ± 0.5 ºC. **A.-F.** Postflexion larva (17, 18, 19, 21, 23, 25 DAH). **G.** End of metamorphosis (29 DAH). **H.** Juvenile stage (30-39 DAH). Scale bars: 1 mm.

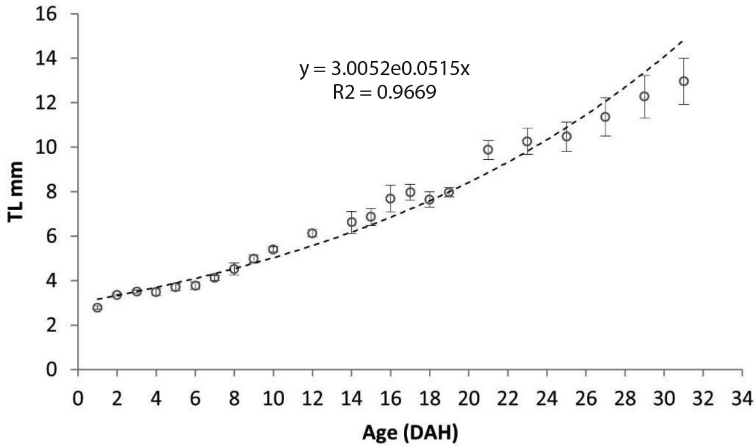

Fig. 5. Total length-age relationship through the larval development stage of black neon tetra larvae.

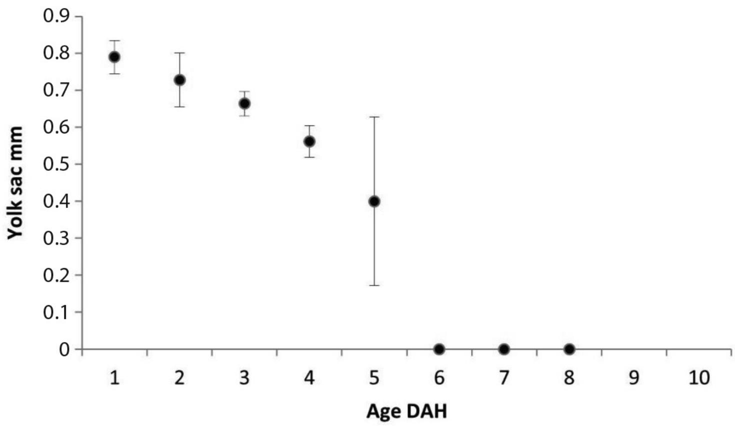

Fig. 6. Yolk sac length of black neon tetra larvae from 1 DAH to 5 DAH (mm).

assume a two nodular sac in appearance following the 14-15th days. The observations on the study showed that the main swim bladder began to inflate on the 3rd day. Therefore, the longest total length the more the length of the swim bladder continued. The reason why the swim bladder decreased in length on the 14-15th days is because the second swim bladder was inflated (Fig. 7). When the second nodule was formed by the main swim bladder, following which the second nodule as the swim bladder continued to extend backwards to the body on the next days until the juvenile phase started (Fig. 8). The size of the swim bladder increased proportionally to the total length (TL) of the larva.

The present study determined that broodstocks of black neon tetra spawned at dawn few hours before sunrise. Spawning lasts for a few hours. Incubation period of eggs is quite short. Eggs hatch several days after fertilization, which means that the embryonic development phase has been completed in 20.00-22.00th hour. The main processes of metamorphosis could be summarized as follows; One observed that the initial swelling of the swim bladder occurred on the 3rd day after hatching while mouth and anus opening and free swimming was on the 4-5th days (Fig. 9). The yolk sac was depleted on 6 DAH. The appearance of caudal fin rays and differentiation of the anal and dorsal fins emerged in the 10th and 14th

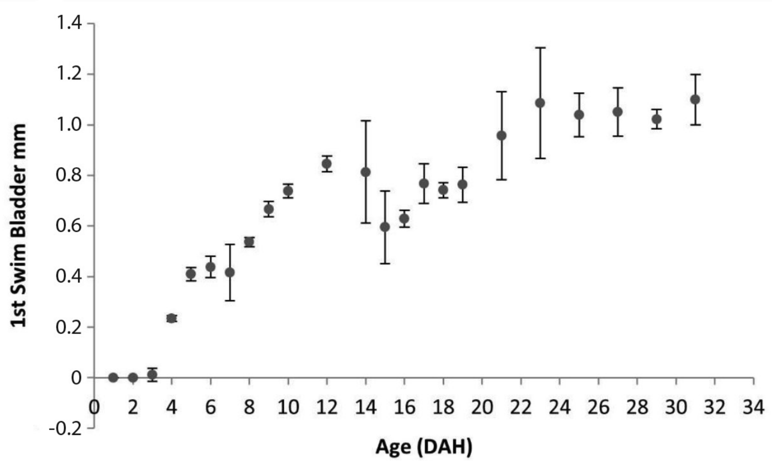

Fig. 7. Length data from the first inflation of the first swim bladder to the juvenile stage (mm).

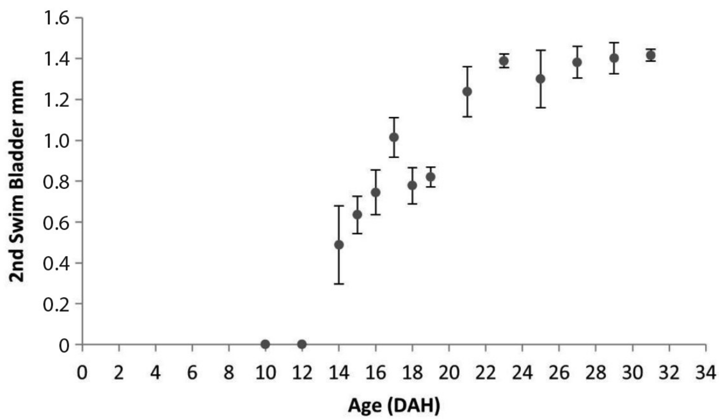

Fig. 8. Length data from the first inflation of the second swim bladder to the juvenile stage (mm).

days, respectively. Flexing of the notochord end called. Flexion was observed on 12-13th days (Fig. 9). Differentiated anal and dorsal fin rays became prominent on the 15th day. The second swim bladder caused by the second nodule was seen on the 14-15th days (Fig. 9). On the 20-21st days, the adipose fin was determined to be visible for the first time. The beginning of the caudal fin bifurcation was recorded on the 18th day, following which juvenile phase was observed to be reached on the 29-30th days when larval stage was over (Fig. 9).

Anatomical observations: On the 1st day, the digestive system of the yolk sac larva was in the shape of a flat long tube (Fig. 10a). The swim bladder dorsal to the yolk sac was about to be in the process of development. Early on the 3rd day, the mouth was open, and the swim bladder swollen with the digestive canal obviously in the form of a tube (Fig. 10b). The yolk sac was depleted on the 5th day, and the stomach and intestine developed instead (Fig. 10c).

The larva can ingest and digest exogenous food (live feed / *Artemia*). On the 10th day, the liver replaced the yolk sac with stomach and intestinal folds increasing (Fig. 11a). The first swim bladder dorsal to the digestive system extended to the posterior (Fig. 11a). On the 19-20th days, the development of the second

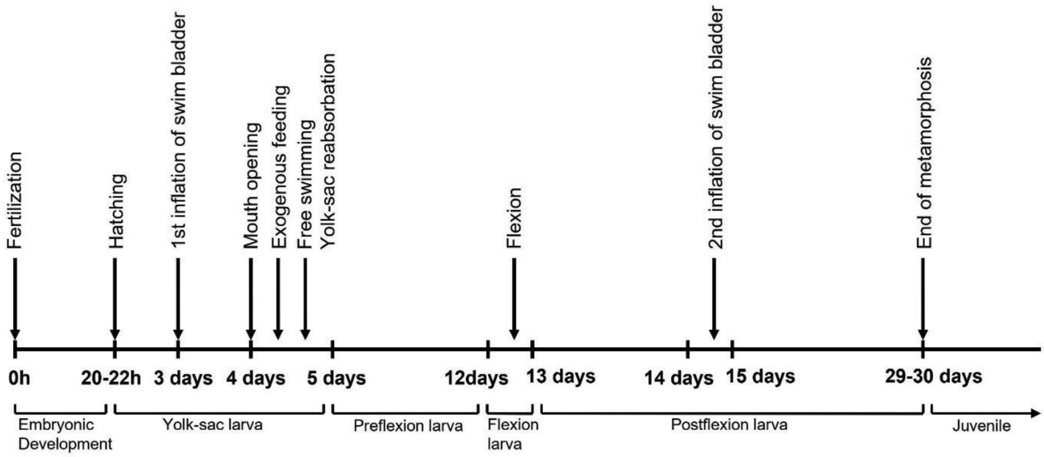

Fig. 9. The main events of larval development in black neon tetra (*Hyphessobrycon herbertaxelrodi)*, 24 ± 0.5 ºC.

swim bladder was completed (Fig. 11b). The swim bladder could be seen as in a double nodular form by histological evidence (Fig. 11b). The stomach and intestine of the larva were in a form or a structure similar to those of juvenile individuals in those days when powder could be digested. Large food particles were observed in the stomach and intestine (Fig. 11b, Fig. 11c). The body depth increased. 29-30 DAH; Metamorphosis was completed and the larvae have thoroughly grown into juveniles.

DISCUSSION

Numerous fish species have the capacity to spawn once a year whereas tetra species can spawn many times all the year round (Cole et al., 1999). They are defined as egg laying species in reproductive behavior and do not assume parental care (Hill & Yanong, 2002). Eggs of tetra species are classified as slightly slimy sticky and sinking ones (Cole et al., 1999; Cole & Haring, 1999). The present study observed that larvae of black neon tetra hatched 20-22 hours after fertilization. Females of black neon tetra are bigger than its males. Water-related parameters essential for them to reproduce is that water be in softness to intermediate hardness of 100 ppm in general, temperature 22-27 °C and pH 7.5 (Çelik et al., 2012). Spawning occurs at dawn just hours before sunrise as in other tetra species, followed by fertilization, then hatching of embryos within the 22-26 h period (Goslawski, 1981; Kornobis, 1990; Romig, 1995).

The studies on *Gymnocorymbus ternetzi* (Çelik et al., 2012) and serpae tetra *Hyphessobrycon eques* (Çelik & Cirik, 2020) species, both of which are from the same family as in the black neon tetra showed that their embryonic phases of development were completed in 20-22 hours, which applies for black neon tetra as well. On the other hand, metamorphosis phenomena of larval development processes can be said to occur in the three tetra species at about the same periods of time. For example, initial swelling of the swim bladder occurred in serpae tetra and black neon tetra on the 3rd day while it was seen in *G. ternetzi,* on the 2nd day (Çelik et al., 2012; Çelik & Cirik, 2020). Opening of mouth and anus and beginning of free swimming in black tetra species ensued on the 3rd day and in serpae tetra and in black neon tetra species on the 4th day (Çelik et al., 2012; Çelik & Cirik, 2020). It is interesting to note that depletion of yolk sac showed differences in the three tetra species. It was observed that black tetra larva depleted the yolk sac in 3 DAH (Çelik et al., 2012), serpae tetra larva in 4 DAH (Çelik & Cirik, 2020) and black neon tetra in 6

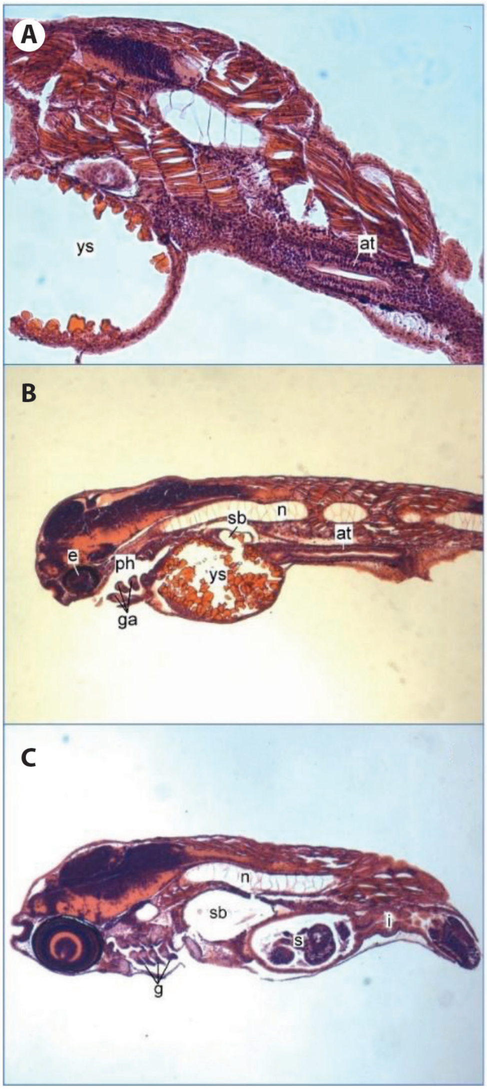

Fig. 10. Sagittal sections of black neon tetra larvae. **A.** 1 DAH (BX51: Olympus™, 100×). **B.** 3 DAH (BX51: Olympus™, 40×). **C.** 5 DAH (BX51: Olympus™, 40×). *at* alimentary tract; *e* eye; *g* gill; *ga* gill arches; *i* intestine; *l* liver; *n* notochord; *oe*, oesophagus; *ph* pharynx; *s* stomach; *sb* swim bladder; *t* teeth; *ys* yolksac.

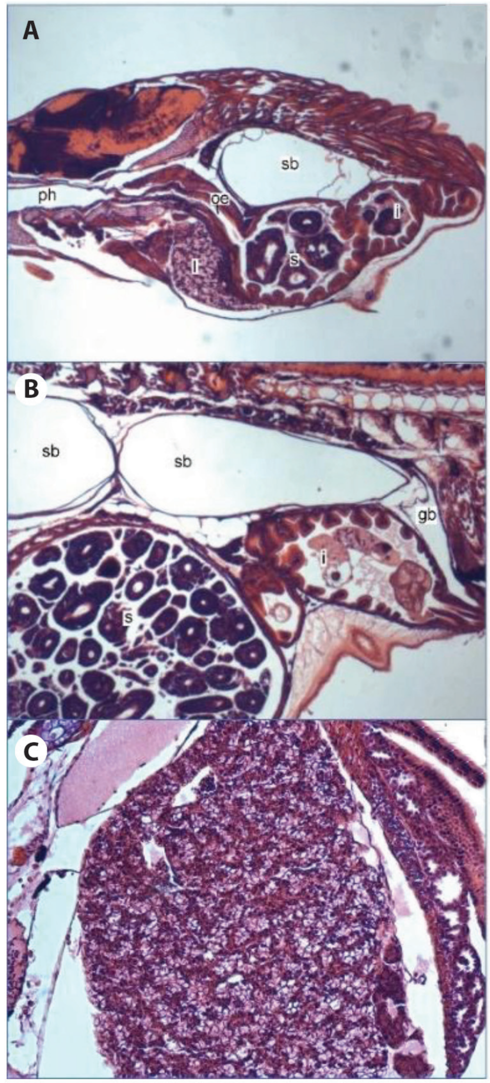

Fig. 11. Sagittal sections of black neon tetra larvae. **A.** 10 DAH (BX51: Olympus™, 40×), **B.** 19 DAH (BX51: Olympus™, 40×). **C.** 24 DAH (BX51: Olympus™, 100×). *gb* gall bladder; *i* intestine; *l* liver; *oe* oesophagus; *ph* pharynx; *s* stomach; *sb* swim bladder.

DAH (the present study). Developmental processes of the three tetra species exhibited that appearance of caudal fin rays and differentiation in anal and dorsal fins occurred in black tetra and serpae tetra on 11-12 DAH (Çelik et al., 2012; Çelik & Cirik, 2020) but in black neon tetra on 10-14 days (the present study). The process of the notochord end flexing, briefly called flexion occurred in black tetra (Çelik et al., 2012) and black neon tetra (the present study) in 10-12 days in and serpae tetra (Çelik & Cirik, 2020) in the 13 days. Differentiated anal and dorsal fin rays in black neon tetra formed 3 days later than the other two tetra species on the 18th day and in black tetra and serpae tetra on the 15th day. Development of the second swim bladder related to the main bladder occurred in the three tetra species almost at the same time (15-17 DAH). The first appearance of the adipose fin was observed in black tetra in 22-23 days (Çelik et al., 2012) in serpae tetra in 24th-25th days (Çelik & Cirik, 2020) and in black neon tetra in 20-21st days (the present study). Bifurcation of caudal fin was found to begin in black tetra and serpae tetra in 19th day (Çelik et al., 2012; Çelik & Cirik, 2020) in black neon tetra in 18th day (the present study), following which the end of the larval process followed by reaching the juvenile phase were seen to be completed in serpae tetra, black neon tetra and black tetra in the 28th, 29th and 29-30th day, respectively. According to metamorphosis processes in larval development phases in the three tetra species different in morphological appearance, their development phases can be cited to be quite similar to each other. There were variations between the periods of time when some morphological processes occurred. However, a general assessment of the larval development process can suggest that these three species develop similarly to each other, from which one can draw a general conclusion on larval development processes of all the other tetra species.

During the larval development of teleosts, the digestive system ontogenesis (the ontogenesis of the digestive system) was defined in three ways such as agastric, precocial and altricial (Rønnestad et al., 2013). Agastric fish species (Cyprinidae, Gobiidae) depends on alkaline and pancreatic proteases for hydrolysis of protein in the entire life cycle of the fish. As the larva grows, digestive function of the intestine increases based on its length, therefore the digestive capacity of the larva would increase as well (Dabrowski & Poczyczyński, 1988). Larvae of Precocial fish species (Salmonidae, Bagridae) develop fully functional stomachs with acid protease activity prior to exogenous feeding, which implies that precocial species increase their digestive capacity significantly more than agastric fish before they are fed with microparticle feeds exogenously (Yang et al., 2010). Finally, altricial species exhibit a gastric larvae characteristic until the time when the stomach differentiates and digestion begins with the protease activity increasing due to alkaline protease process in their initial feeding (Sparidae, Paralichthyidae) (Faulk & Holt, 2009; Santamaría et al., 2004). Successful transition from live to microparticle feeds in the larva breeding of altricial species is mostly based on the differentiation of a functional stomach (Faulk & Holt, 2009; Rønnestad et al., 2013; Thompson et al., 2019). Therefore, awareness of when the stomach differentiates in the process of larval development is important.

Likewise, it is of great importance to know the processes such as opening of mouth, depletion of the yolk sac and development of the digestive system in the larva breeding business. Larva of black neon tetra examined in the present study is also included in the class of altricial larva. It has been similarly reported that the larvae of some tetra species such as black skirt tetra *G. ternetzi* (Çelik et al., 2012), serpae tetra *H. eques* (Çelik & Cirik, 2020) and neon tetra *Paracheirodon innesi* (Lipscomb et al., 2022) are altricial. It is not appropriate to supply these species exogenously with microparticle feed until digestion in their stomachs has been completely functional by then they are supposed to be fed with live feeds like rotifers and *Artemia*. Larvae of neon tetra, *P. innesi* species complete their stomach development between 12 and 20 days after the hatching finally become functional in digestive sense (Lipscomb et al., 2022). In the present study, larvae of black neon tetra were observed to similarly complete their stomach development between the 10 and 19th days following the hatching, according to which it is recommended that larvae of the tetra species hereby cited should not be supplied with microparticle feeds before 19–20th days in the larva breeding process. Altricial species can be said to develop faster and thus have higher survival rates than precocial species (Nakatani et al., 2001; Santos et al., 2020). The consequences

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of the present study are consistent with those of the trials made on some characin species such as neon tetra *P. innesi* (Lipscomb et al., 2022), black skirt tetra *G. ternetzi* (Lipscomb et al., 2020) and black prochilodus *Prochilodus nigricans* (Souza da Silva et al., 2022) but nevertheless, more comprehensive studies can be conducted in this field anyway.

Mouth and anus opening with the pigmentation of eyes are those processes that occur simultaneously in the phase of preflexion (Cajado et al., 2021; Oliveira et al., 2021; Silva et al., 2021), all of which are of course related to the fact that larvae are fed exogenously. Accordingly, larvae of black neon tetra began to feed exogenously from the 4th day after mouth and anus opening, when they were inevitably supplied with *Artemia* since their digestive system did not develop completely. Transition from endogenous to exogenous feeding is of critical importance considering the success of larviculture (Abdo et al., 2015), because if larvae capable of surviving thanks to their yolk sacs in earlier days after hatching were not fed exogenously due to depletion of them, they could encounter autophagy towards their own tissues that we can described as self-digestion (Souza da Silva et al., 2022) which was thoroughly observed by Ferreira et al. (2009) who studied larvae of *Glossolepis incisus* (Melanotaeniidae, Atheriniformes).

Pigmentation in fish larvae is the basic taxonomic criterion used to identify them in their natural habitats and under controlled breeding conditions (Lima et al., 2020; Santos et al., 2020). It would thus be useful to know pigmentation processes which occur in larval development phases of fish species. The pigmentation process from the hatching of black neon tetra until its juvenile was observed in the present study therefore proving that larvae assumed an appearance of typically mature individuals after 29-30 days, which was reported to be the same in similar tetra species as well (Çelik et al., 2012; Çelik & Cirik, 2020). However, color-related anomalies could be widely seen in fish larvae, which inevitably leads to losses of yield and quality in fish breeding business. Anomalies observed in pigmentation are the very problem in almost all fish species in aquaculture (Cal et al., 2018), which on the other hand proves more problematic in the ornamental fish sector since ornamental fish are organisms bought and sold just because of their beauty and physical forms. Therefore, coloration of ornamental fish such as black neon tetra is supposed to be closely monitored from the early periods of time of the process.

Formation of fins during early ontogenesis depends on development of swimming skills which is of vital importance in terms of influences on larval behavior and growth (Portella et al., 2014). Fins are the organs capable of increasing likelihood of larval survival as well as strengthening movement and distribution of larvae in water (Silva et al., 2021). It is therefore of great use to follow the development processes of fins in the larval period. In the present study, it was recorded that larvae of black neon tetra had well developed primordial fins in the early days when they hatched and all the other fins, but the pelvic fin did not differentiate yet. Dorsal and anal fins began to differentiate in the 11-13th days when tail fin rays further developed to be prominent. Dorsal anal and tail fins continued to develop in the 23-28th days as well. Fins completed their morphological development after 29-30th days. The larval fin development in the present study was observed to be similar in some black tetra species such as skirt tetra *G. ternetzi* (Çelik et al., 2012), serpae tetra *H. eques* (Çelik & Cirik, 2020) and neon tetra *P. innesi* (Lipscomb et al., 2022) as well.

In conclusion, the morphological processes recorded during the larval development of black neon tetra (*H. herbertaxelrodi*) were reported to exhibit resemblance to those found in the studies on some characin species such as *G. ternetzi* (Çelik et al., 2012), *Astyanax lacustris* (Stevanato & Ostrensky, 2018; Santos et al., 2020), *Brycon amazonicus* (Neumann et al., 2018), *H. eques* (Çelik & Cirik, 2020) and *P. innesi* (Lipscomb et al., 2022). Negligible differences can appear between developmental stages due to such factors as water temperature, medium conditions and feeding/nutrition regime in which the species is bred. Moreover, there can be variations in periods of time in other morphological processes such as mouth opening, yolk sac depletion and free swimming. However, metamorphic events during larval developments of these species are seen to occur similarly. On the other hand, it should be emphasized that larval development processes of characin species used as ornamental fish are different from those of Cichlidae family species including *Cichlasoma dimerus* (Meijide & Guerrero, 2000), *Astronotus ocellatus* (Paes et al., 2011), *Labidochromis caeruleus* (Saemi-Komsari et al., 2018), *Iodotropheus sprengerae* (Çelik et al., 2022).

The histological and morphological findings revealed during the larval development stages of the black neon tetra may provide insights for professional businesses engaged in the commercial production of this species. The increased success of larval production in aquaculture is directly related to the economic aspects of the business. In this way, several positive contributions can be made, such as increasing larval survival rates, enhancing growth rates, and reducing stress levels. Therefore, the findings shared in this study may be significant to commercial aquaculture operators.

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