The Israeli Journal of Aquaculture – Bamidgeh • ISSN 0792-156X • IJA.74.2022.1821652, 10 pages
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Embryonic Development of Black Neon Tetra *Hyphessobrycon herbertaxelrodi* Géry, 1961

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(Received Nov 10, 2022; Accepted Nov 24, 2022; Published Dec 03, 2022)

Keywords: Black neon tetra, characidae, embryonic development, *Hyphessobrycon herbertaxelrodi*, ornamental fish

Abstract

The current study described the embryonic development stages of black neon tetra (*Hyphessobrycon herbertaxelrodi*), an economic ornamental fish. We characterized the stages of the zygote, cleavage, blastula, gastrula, segmentation, *pharyngula,* and hatching occurring during embryogenesis, which emphasizes changing spectrum of the main development processes from fertilization to incubation. The findings were put forth and photographed by examining live embryos under microscopy. Embryonic development of black neon tetra concluded at 24 ± 0.5°C water temperature at 20-21 hours. The first embryonic division occurs within the first 43 minutes after fertilization, and the process goes on to blastula at 02 hours and 28 minutes. The gastrula stage began at 02.57 hours, while 6 somite segmentation stages were observed to occur at 08.14 hours. Following the *pharyngula* stage seen between 17 to 20 hours, the hatching occurred at 20-21hours. The results of this study can provide significant benefits to professional breeders in the aquaculture of black neon tetra and other ornamental fish species.

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Introduction

The global aquarium fish industry is a multibillion-dollar business, with an annual legal trade volume of about 15-20 billion dollars (King, 2019; Pouil *et al.,* 2020). There are thousands of fish species in this business network. The Characidae genus is valuable species in the industry (Freitas & Rivas, 2006). The black neon tetra is one of the most popular Characidae species in the worldwide ornamental fish market and aquarium industry (Tavares, 1997; Park *et al.*, 2014). Black neon tetra (*Hyphessobrycon herbertaxelrodi*) is a famous freshwater aquarium species. (Gimeno *et al*., 2016). Soft in character and omnivorous in nourishment, *H. herbertaxelrodi* inhabits rivers and lakes, preferring to swim in groups and feed on worms, crustaceans, and plants (Zhang *et al.,* 2020). The study examined the embryonic developmental stages of *H. herbertaxelrodi* grown under laboratory conditions.

It is essential to study its embryonic and larval development both for taxonomic purposes and for captive cultivation, especially in determining when yolk sac absorption and mouth opening occurs, which indicates the necessity for exogenous feeding (Sato *et al.,* 2003). Such studies can provide important information relevant to developmental, hatching success, larval feeding, growing, and weaning stages in teleost development (Zadmajid *et al.,* 2019). Successful larval rearing and assessment of larval quality depend significantly on the information regards to larval size, duration of embryonic developmental stages, consumption time of the yolk sac and larval development stages of the cultured species, mouth gape, first feeding and swimming pattern of the larvae (Çelik & Cirik, 2020; Gomathi *et al.,* 2021). A detailed study about the embryonic development of black neon tetra is not in the literature. Therefore, the results of this study are remarkable.

Materials and Methods

In this study, one-year broodstock individuals of black neon tetra were used. Fishes were specifically nurtured with commercial ornamental fish feeds (Tetramin Granulat, Tetra, Germany; Protein: 46%, Oil: 12%, Fibre: 3%, Ash: 11%, Moisture: 8%) three times a day. During care and preservation of the broodstock, water temperature, pH and conductivity were monitored daily at 24 ± 0.5°C, 6.0 - 6.5, and 100 - 200 µS, respectively. Water temperature was controlled by additional submerged heaters (100 watts). The photoperiod was maintained at 11L/13D by fluorescent lighting (lights were kept on from 07:00 to 18:00 hours). Broodstocks were preserved in 40 L glass aquaria. Randomly selected from among the broodstock in the tank, three pairs (3 males and 3 females) were transferred into another spawning container of 15 L in the late afternoon. Spawning lasted for 1-3 h on the following day and was observed around dawn time.

Fertilized eggs were collected soon after the spawning and maintained in aquaria at 24 ± 0.5 °C. Some were carried into a beaker (500 mL) to observe their embryonic development, whereas others were kept in 15 L aquaria at 24 ± 0.5 °C. The eggs were observed from spawning until hatching via an Olympus BX51 research microscopy (Hatagaya, Shibuya-ki, Tokyo, Japan) and photographed by a color video camera (Q Imaging, Micropublisher 3.3 RTV, Burnaby, BC, Canada). According to Kimmel et al. (1995), stages of embryonic development were identified.

The specimens were studied via an Olympus SZX7 zoom stereomicroscope and photographed by a color video camera then the result that the diameters of eggs were measured using the image analysis program (Q Capture Pro, version 5.1.1.14, Dendermonde, Canada).

In this study, neither any chemical nor the eggs were killed. Just the eggs were observed under research microscopy and photographed. Therefore, ethical approval was not obtained for this study because it was not required.

Results

Egg diameters of the black tetra fish are between 901 to 927.68 μm with a mean of 912.38 ± 7.41 μm (n = 15). The yolk sac is brownish in color, the shape of the egg spherical, and its external shell translucent, sticky and demersal.

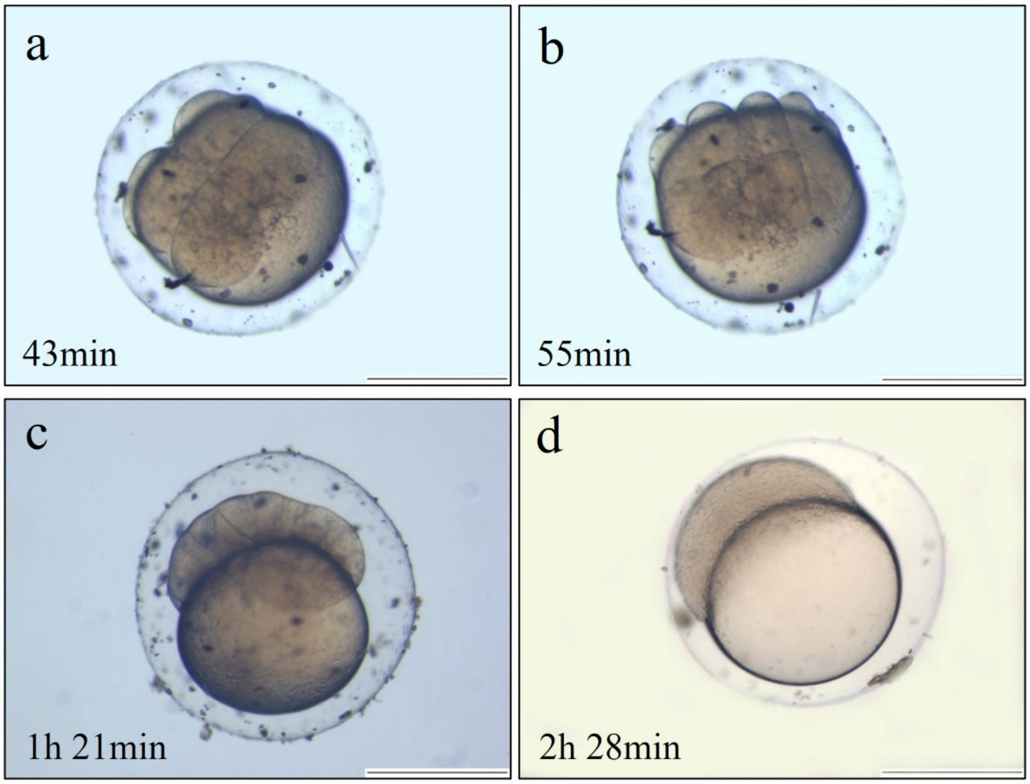

Figure 1 Embryonic development stages of black neon tetra species: (a) 4-blastomere stage; (b) 8-blastomere stage; (c) 64-blastomere stage; (d) High stage. The scale is 500 microns.

When the second division at the animal pole was seen to emerge after 43 minutes, the blastomere had been divided into four (4) equal pieces (**Figure 1a**). The third division (55min), defined as the 8-blastomere stage, was observed to have the cells in horizontal sequences and 2X4 form (**Figure 1b**). **Figure 1c** shows the 64-blastomere stage to include lessened (minimal) and irregular cellular dimensions in shape, with the blastula stage beginning to appear. The surfaces of the blastoderm had been lumped as the divided blastomeres lessened/decreased in size 2h 28 min after the fertilization, which is called the high stage (**Figure 1d**).

 • https://doi.org/10.46989/001c.57301

The Dome stage was seen when epiboly started in blastoderm 2h 57 min after fertilization. (**Figure 2a**). The blastoderm was noticed to cover 30% of the egg (3h 24min) in **Figure 2b** (3h 24min). The germ ring appeared 4h 92min after the fertilization in **Figure 2c**. The moment when the blastoderm covered 70% of the egg is presented in **Figure 2d** (4h 55min).

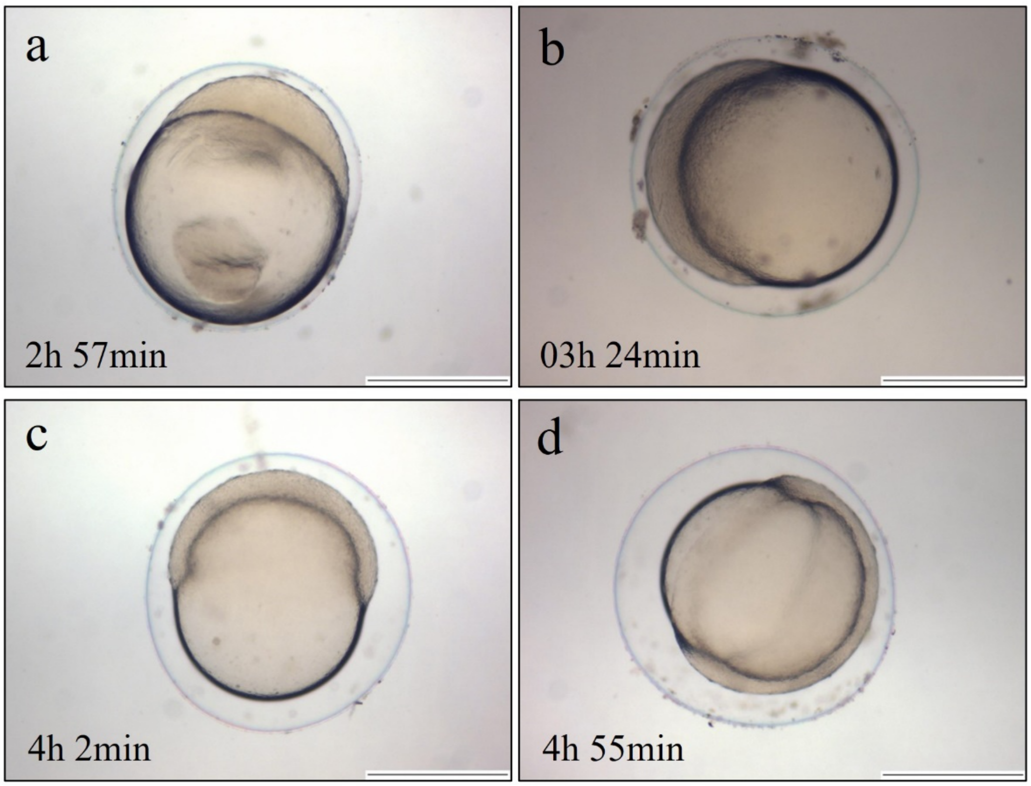

Figure 2 Embryonic development stages of black neon tetra species: (a) Dome stage; (b) 30% epiboly-stage; (c) Germ ring; (d) 70% epiboly-stage. The scale is 500 microns.

The bud stage, when the embryo distended in both its ends to form body and tail buddings, is shown in **Figure 3a** (07.00h). Figure 3b, c, and d exhibit that upon fertilization, the middle section of the would-be vertebral formation was seen to produce the 6, 8, and 16-somite stages at 8h 24min, 9h 19min, and 12h 46min, respectively. The 6-somite stage clearly shows the eye socket to form (**Figure 3b**), the 8-somite stage exhibits the orbital process where the eye would later emerge (**Figure 3c**), and the 16-somite stage indicates the tail beginning to break off the vitellus (**Figure 3d**).

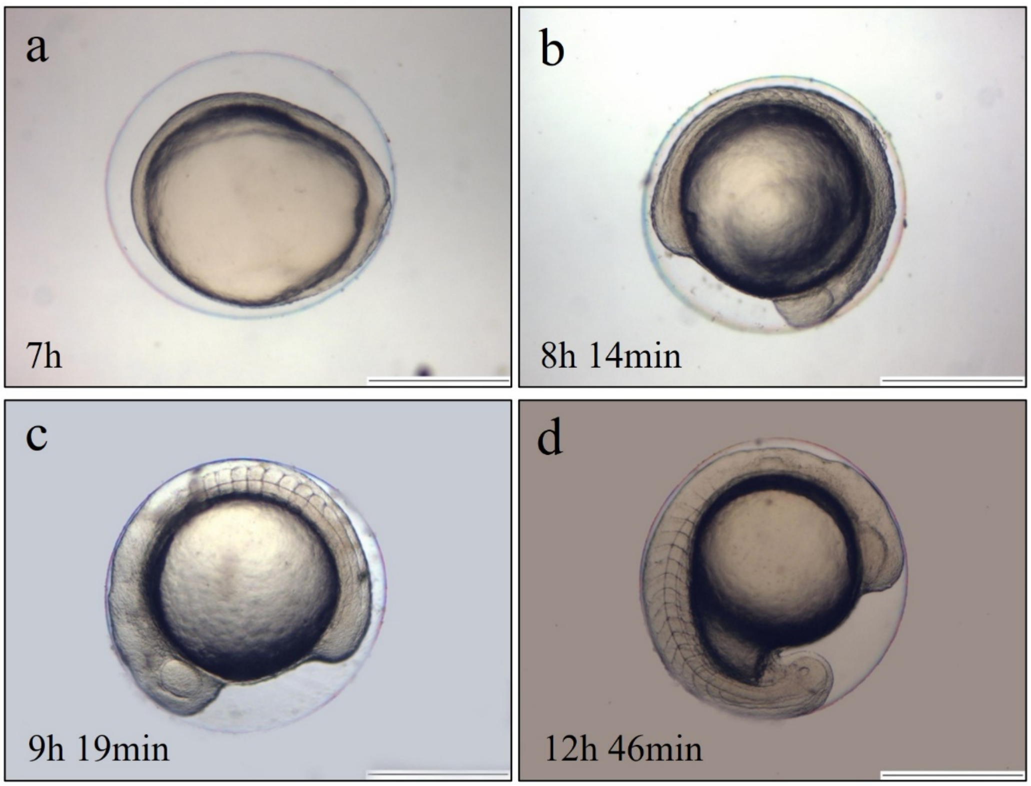

Figure 3 Embryonic development stages of black neon tetra species: (a) Budding stage; (b) the 6-somite stage; (c) the 9-somite stage; (d) the 16-somite stage. The scale is 500 microns.

The 17-somite stage in **Figure 4a** shows the ear capsule forming the hearing organ and the primordial fin to appear (15h 5min)—the hearing capsule formed at the 21-somite stage about 17-20h after fertilization. The embryo began to spin in small jerks (**Figure 4b**). The 26=somite stage showed the two otoliths formed in the otic capsule (20.00h). Muscle movements also increased (**Figure 4c**). The imaging of the individual, which was about to complete its embryonic development and hatch, is seen in **Figure 4d** (21h 10 min).

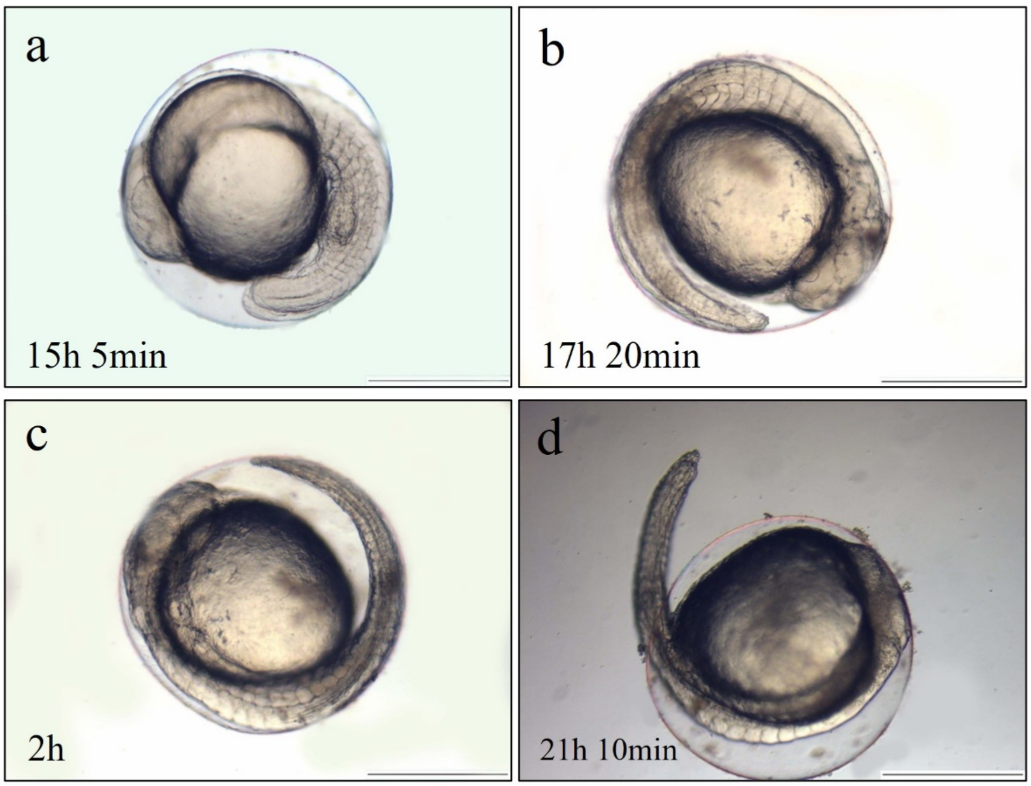

Figure 4 Embryonic development stages of black neon tetra species: (a) the somite stage; (b) the 21 somite stage; (c) the 26 somite stage; (d) hatching. The scale is 500 microns.

Table 1 summarizes significant data about the embryonic development of black neon tetra found by observing the fertilized eggs maintained at 24±0.5°C water temperature. The study described the embryonic development of the laboratory-reared black neon tetra (*Hyphessobrycon herbertaxelrodi*) under controlled aquarium conditions. Embryonic development completed in 20 to 21h hatching post fertilization (hpf).

Table 1 Embryonic development stages of black neon tetra (*Hyphessobrycon herbertaxelrodi*) at 24 ± 0.5°C

Main stages	Substages	Time (h:min)	Explanation	Figure
Zygote	4 cells	0:43	In the second cleavage, germinal disk was divided to form 4 blastomeres	1a
	8 cells	0:55	Third cleavage, the 8 blastomere stage	1b
	64 cells	01:21	Sixth cleavages, blastomeres continue to divide but their cell dimensions are less synchronized	1c
Blastula	High stage	02:28	Epibolic cells increase	1d
Gastrula	Dome	02:57	Blastoderm is the initial stage of epiboly	2a
	30% epiboly	03:24	Germ ring has covered 1/3 of the yolk	2b
	Germ ring	04:02	Germ ring has covered half (½) of the yolk	2c
	75% epiboly	04:55	75% of the yolk has been covered by the blastoderm	2d
	Bud stage	07:00	Budding of tail and body	3a
Segmentation	6 somites	08:14	Eye socket begins to form	3b
	9 somites	09:19		3c
	16 somites	12:46	The tail begins to leave the vitellus	3d
	17 somites	15:05	The ear capsule to form the hearing organ begins to appear	4a
Faringula	21 somites	17:20	Embryo starts to spin in small jerks	4b
	26 somites	20:00	Significant muscle movements are observed	4c
Hatching		21:10		4d

Discussion

Such species associated with black neon tetra (*H. herbertaxelrodi*) as *Hydrocynus vittatus* (Steyn *et al.,* 1996), *Gymnocharacinus bergi* (Cussac & Ortubay, 2002), *Brycon gouldingi* (Faustino *et al.*, 2018), *Gymnocorymbus ternetzi* (Çelik *et al.,* 2012), *Paracheirodon innesi* (Vilasrao, 2013) and *Astyanax altiparanae* (dos Santos *et al*., 2016) are known to have eggs with diameters in the range of 0.65-1.5 mm. However, tetra species such as *Brycon nattereri* (Maria *et al.*, 2017) and *Brycon orthotaenia* (Gomes *et al.,* 2011) were found to include eggs with a diameter of 1.5-3.09 mm.

Laboratory experiments performed with some Characidae species showed that temperatures between 21 and 27°C are optimal for the embryos in the development process from the fertilization stage to the hatching stage. Those performed with *Astyanax altiparanae, Brycon gouldingi, Brycon orthotaenia, Gymnocharacinus bergi, Gymnocorymbus ternetzi, Hydrocynus vittatus, Hyphessobrycon anisitsi, Hyphessobrycon eques,* and *Paracheirodon innesi* mainly exhibited almost the exact impacts of temperature on early stages of life (dos Santos *et al*., 2016; Faustino *et al.*, 2018; Gomes *et al.,* 2011; Cussac & Ortubay, 2002; Çelik *et al*.*,* 2012; Steyn *et al.,* 1996; Park *et al*., 2015; Park *et al*., 2014; Vilasrao, 2013).

Many fish species show that blastomeres are regular in size and shape (Hall, 2008). Such a property of black neon tetra is similar to black skirt tetra (*Gymnocorymbus ternetzi*) (Çelik et al., 2012) and serpae tetra (*Hyphessobrycon eques*) (Çelik & Cirik, 2020).

The hatching periods in some Characidae species closely related to black neon tetra are similar to those in each other (Park *et al*., 2014; Park *et al*., 2015). Hatching times vary at different temperatures but egg opening periods range between 11 and 38 hours (Vilassrao, 2013).

Although the egg size of the ornamental fish species could be widely variable, most have a diameter of about 0.8 mm (Watson & Chapman, 2002). Egg quality is known to be proportional to larval survival. The current study found the egg diameters of black neon tetra to be around 901 – 927.68 µm with an average diameter of 912.38 ± 7.41 µm (n = 15). Other studies reported that the egg diameter of Hypesssobbrycon serpae (Characidae) included in the same family as black neon tetra (*Hyphessobrycon herbertaxelrodi*) was in the ranges of 0.74-0.90 mm (Cole & Haring, 1999), 0.91–0.93 mm (Park et al., 2014) and 847.16–1040.29 µm (Çelik & Cirik, 2020).

In conclusion, morphological evidence indicates that embryonic development stages in black neon tetra are the same as in other characins (Romagosa *et al.*, 2001; dos Anjos & dos Anjos, 2006; Pan *et al.*, 2008; Faustino *et al*., 2012; Çelik *et al*., 2012; Faustino *et al.,* 2018). Fertilized eggs of black neon tetra individuals are spherical, transparent, demersal, and adhesive. The embryonic development stage was completed at 20-21 h. The patterns of cleavage in black neon tetra is the same as those in other characin species (Romagosa *et al*., 2001; Faustino *et al*., 2011, Faustino *et al*., 2018; Çelik *et al*., 2012; Çelik & Cirik, 2020). These findings may provide a basis for further studies to determine the early development stages of black neon tetra and like ornamental fish species.

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Authors' contributions

Pınar Çelik: Designed the experiments, collected the data for this study, collaborated in interpreting the results, and wrote the initial draft of this manuscript.
İhsan Çelik: Developed the original hypotheses, conducted the statistical analyses, collaborated in interpreting the results, and finalized the manuscript.
All authors have read and approved the finalized manuscript.