



## RESEARCH ARTICLE

# Larval Development of Penguin Tetra (*Thayeria boehlkei*): Morphological Observations

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## ABSTRACT

In this study, the larval development stage of the penguin tetra fish (*Thayeria boehlkei*), a popular species in the freshwater aquarium fish industry, was investigated. For this purpose, penguin tetra larvae were morphologically observed from time to hatching until they reached the juvenile stage. Throughout the larval development phase, samples were randomly taken daily and captured on a camera using a stereomicroscope. Larval development stages were categorized into four periods: (I) Yolk-sac larva, (II) Pre-flexion larva, (III) Flexion larva, and (IV) Post-flexion larva. Larvae were fed with *Artemia nauplii* until they reached the juvenile stage for the entire research. Morphological changes, including the state of the yolk sac from hatching, mouth opening, changes in body shape and color, and fin formation processes, were recorded daily. According to the findings, the mouth opened on the 5th day, and external feeding also started on the 5th and 6th days. At the same time, free swimming movements were observed in the larvae, and it was determined that the yolk sacs were consumed. The flexion larva stage occurred on the 15-16<sup>th</sup> days. The larval development process of the penguin tetra fish was completed within 25 days after hatching. After this stage, the juveniles became morphologically identical to their parents.

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**1. Introduction**

The global aquarium fish trade has turned into a multi-billion-dollar industry and continues to grow every year. According to a report published three years ago, the global aquarium fish market size was valued at USD 4.5 billion in 2020 and is expected 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 (Helfman et al., 2009). Penguin tetra is in high demand by aquarium enthusiasts due to its

characteristics such as schooling behavior, unique colorful body patterns, small size, and adaptability to planted aquariums (Axelrod, 1996). It is a popular species among freshwater aquarium species (Weitzman & Palmer, 1997).

The penguin tetra (*Thayeria boehlkei*) is a freshwater fish species native to the Amazon basin, belonging to the Characidae family (Weitzman & Palmer, 1997). In nature, this species is distributed in rivers with dense vegetation (Géry, 1977). Penguin tetras are among the popular species in the aquarium sector due to their attractive colors and swimming behaviors (Axelrod et al., 1967). The popularity of this species in the aquarium trade can put pressure on natural fish stocks.

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To alleviate this pressure on natural populations and make this business sustainable, it is necessary to develop aquaculture practices (Monticini, 2010).

Morphological examination of the larval development of this tetra species is important for understanding the reproductive biology of the species and increasing the success in aquarium fish farming. The larval development stage is a critical period from hatching until the transition to the juvenile stage. During this period, newly hatched larvae undergo morphological and physiological changes until they become juveniles (Fuiman & Werner, 2009). The changes that occur at this stage are directly related to the survival and growth rates of the larvae (Houde, 1987).

In our literature review, we noticed that information on the larval development of penguin tetras is limited. No detailed scientific study on this subject has been found. Understanding the morphological changes during the larval development stage in every aspect will help us better understand the reproductive biology of the penguin tetra and increase the efficiency of the techniques applied in the cultivation of such species (Nakatani et al., 2001). In addition, the findings obtained in this way will allow a better understanding of the larval development of other species in the Characidae family and enable interspecies comparison (Oliveira et al., 2008).

The aim of this study is to examine the larval development stage of penguin tetra fish morphologically from hatching until the transition to the juvenile stage. Within the scope of the study, penguin tetra larvae were photographed daily from the first day they hatched until they reached the juvenile stage. The findings obtained enabled the definition of the larval development stage of this species and the determination of the morphological changes that occur during this period.

## 2. Materials and Methods

### 2.1. Broodstock Management and Larval Rearing

Mature individuals of the species, aged over one year, were utilized for the controlled reproduction in laboratory conditions. Female and male broodstock were maintained separately in 40 L glass aquaria (dimensions: 40 cm length × 30 cm width × 35 cm height; water level: 32-33 cm). All broodstock were fed a commercial aquarium diet (Tetramin Granulat, Tetra, Germany; composition: 46% protein, 12% oil, 3% fiber, 11% ash, 8% moisture) three times in a day. A photoperiod of 12 hours light and 12 hours dark was maintained, with lights on from 07:00 to 19:00. Water parameters in the broodstock tanks were kept constant at a temperature of 24±0.5 °C, pH of 6.0-6.5, and conductivity

between 100-200 µs, using 100-watt aquarium heaters for temperature regulation. For spawning, three females and three males were randomly selected from the broodstock aquaria and transferred to 15 L glass production tanks. Spawning occurred overnight, and the eggs were deposited on the bottom of the tank. After spawning, the broodstock were removed, and the eggs were incubated in the production tank for larval development monitoring. The eggs were maintained at a constant water temperature of 24±0.5 °C.

### 2.2. Larval Sampling

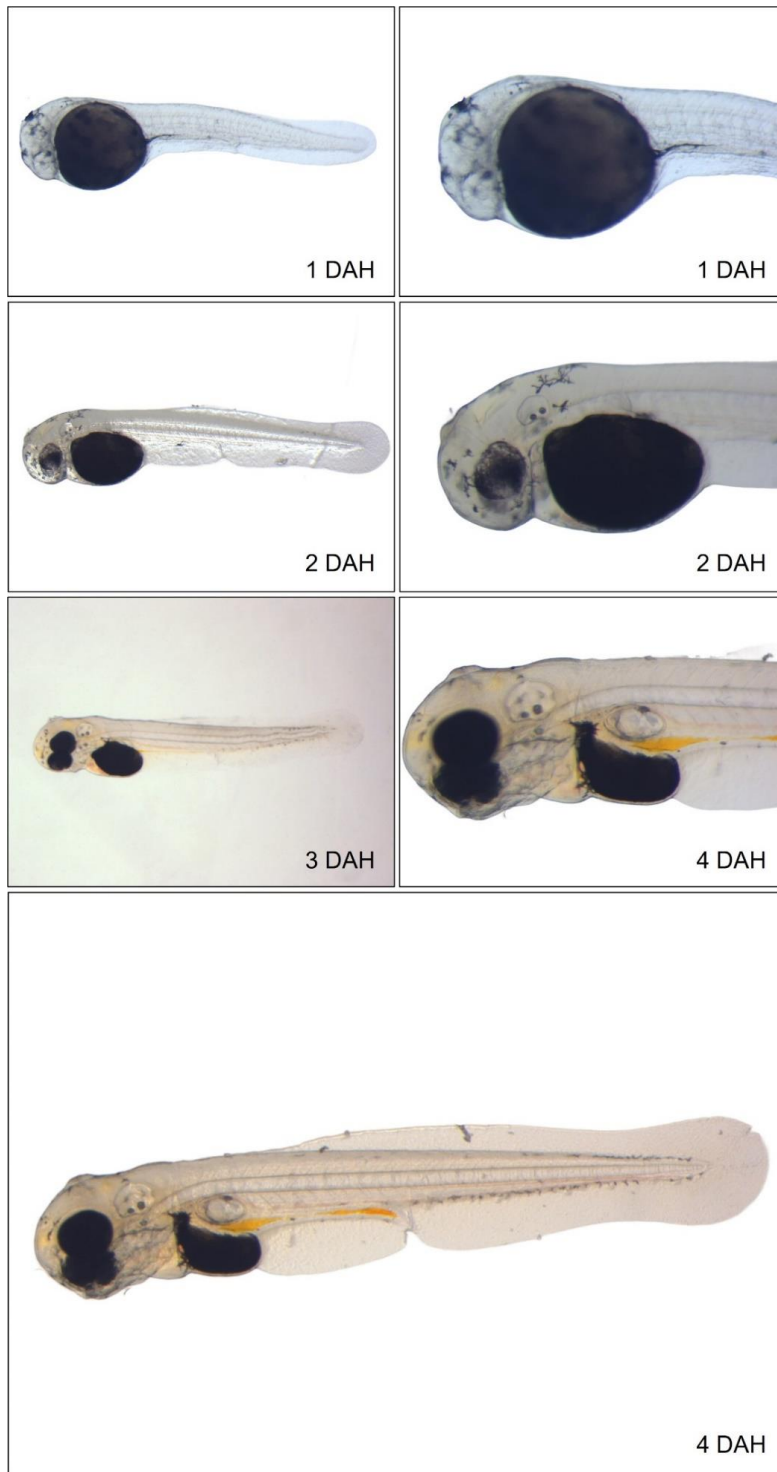
From the first day of hatching until the juvenile stage, random samples of larvae were collected daily. Larvae were observed using an Olympus SZX7 zoom stereomicroscope (Tokyo, Japan) and photographed with a connected video camera (Q Imaging, Micropublisher 3.3 RTV, Canada). Larval development stages were identified according to Kendall et al. (1984) and categorized into four periods: (I) yolk-sac larva, from hatching until yolk sac absorption; (II) preflexion larva, from yolk sac absorption until the start of notochord flexion; (III) flexion larva, from the start of notochord flexion until its completion; and (IV) post-flexion larva, from the completion of notochord flexion until the end of larval development and transition to the juvenile stage. Observations of larval development were terminated once the juvenile stage was reached.

### 2.3. Larval Feeding

Exogenous feeding commenced once the larval mouths opened. Larvae were fed with live *Artemia salina* nauplii (INVE Aquaculture Inc., Dendermonde, Belgium) once daily at a density of 5-10 individuals/mL until they reached the juvenile stage. Artemia cysts were hatched in 1.5 L cylindro-conical plastic containers at a water temperature of 25-26 °C and salinity of 25-30 ppt. Newly hatched Artemia nauplii were used for larval feeding.

## 3. Results

**1 DAH:** On the first day after hatching (1 DAH), the penguin tetra larvae have a large yolk sac (Figure 1). The eyes are in the formation stage, but eye development is not complete (Figure 1). The body is in primordial form, without pigmentation, and has a transparent appearance. From the outside, the digestive system appears to be a straight tube. On the first day, the mouth and anus are closed (Figure 1). There are a few small, scattered pigment spots on the head region. However, the head region also has a generally transparent appearance. Due to this transparency, the otolith structures inside the otic capsule appear as two small dots (Figure 1).



**Figure 1.** Larval development of penguin tetra (*Thayeria boehlkei*). Post-hatching stage (1 DAH), yolk-sac stage, the gas bladder was formed but not filled (4 DAH).

**2 DAH:** The proportion of black pigmentation in the eyes has increased (Figure 1). The eyes are slightly more developed compared to the first day. The head region and body are transparent. A pair of adhesive glands are noticeable on the anterior-upper part of the head region. There is a slight increase in pigmentation in the head region compared to the first day. The formation of the pelvic fins is noticeable. The mouth and anus are closed (Figure 1). The larva makes short-term tail

movements. It cannot swim freely. The yolk sac has become even smaller compared to the previous day. The vertebral column and primordial fin structures can be more easily distinguished from each other. The primordial fin has widened (Figure 1).

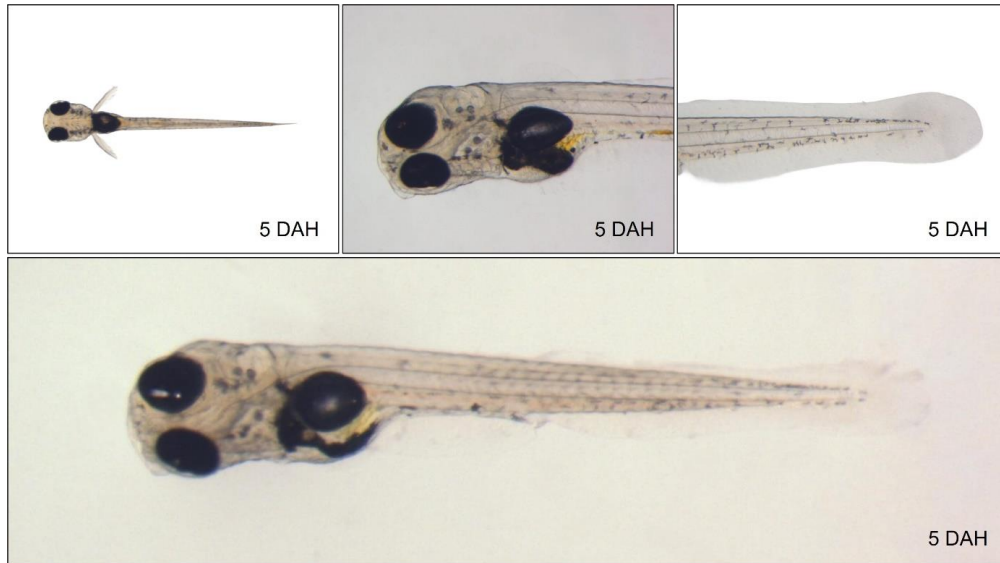
**3 DAH:** Eye development appears to be complete (Figure 1). The head and body are still transparent. The vertebral column and myomere structures are clearly distinguishable.

The yolk sac has shrunk by approximately 2/3 compared to the first day. The mouth and anus are still closed. The digestive system can be seen as a long, straight tube extending to the anus (Figure 1). It can be noticed that the two small dots inside the otic capsule have grown slightly compared to the first days (Figure 1).

**4 DAH:** The yolk sac has become very small (Figure 1). The swim bladder has started to form as a small balloon. The adhesive gland on the anterior-upper part of the head is still present (Figure 1). The larva cannot swim freely. However, the duration of short tail movements has increased. The mouth and

anus are closed (Figure 1). The digestive system is still in a straight structure (Figure 1). It is yellow in color. The notochord tip maintains its straight form. The body is transparent (Figure 1). Pigmentation continues to increase in the form of small black dots throughout the body.

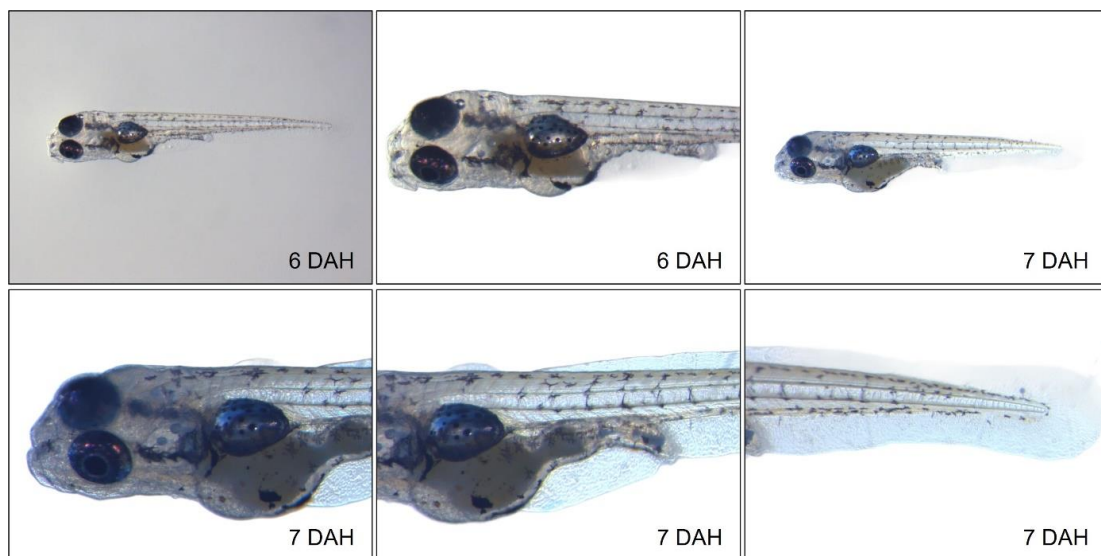
**5 DAH:** The mouth has opened (Figure 2). The yolk sac is depleted (Figure 2). The larva has started free swimming movements. It can take external food during these days. The swim bladder has grown slightly. The notochord tip is not curved (Figure 2). The dorsal, anal, and caudal fins have not yet formed.



**Figure 2.** Larval development of penguin tetra (*Thayeria boehlkei*). Consumption of yolk-sac (5 DAH); opened-mouth stage (5 DAH); exogenous feeding stage.

**6 DAH:** The larva continues to take external food (Figure 3). The body is generally transparent, but the distribution areas of pigmentation continue to expand. Ingested Artemia can be seen in the stomach. Tail formations are not complete (Figure

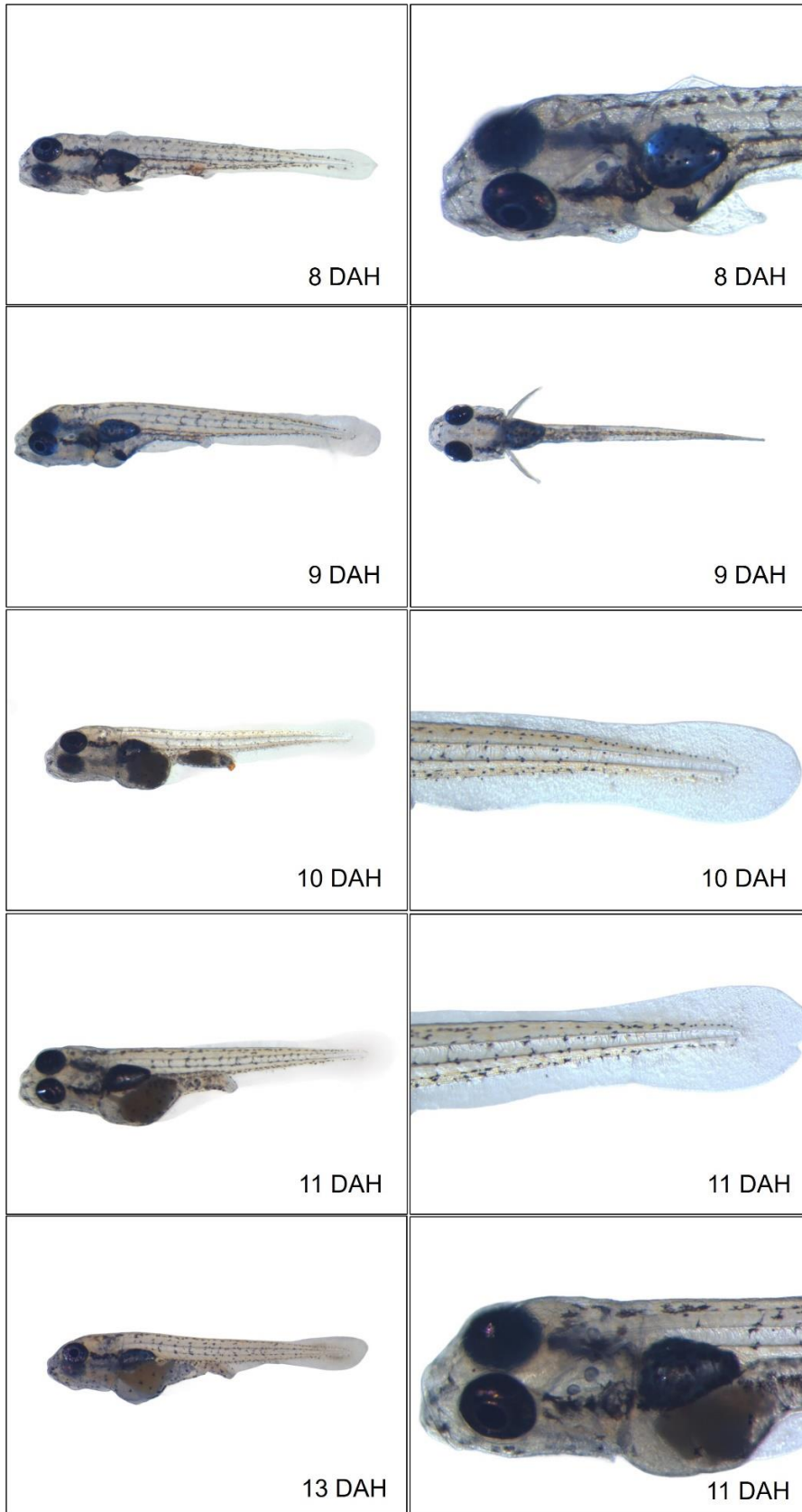
3). The vertebral column is in a straight form. The notochord is not curved (Figure 3). The swim bladder is slightly elongated oval-shaped towards the back of the body.



**Figure 3.** Larval development of penguin tetra (*Thayeria boehlkei*). Preflexion larva stage (6-7 DAH).

**7-14 DAH:** During these days, the larva can swim freely in series. They feed on live food. The density of pigmentation in their bodies increases slightly every day (Figure 3). The dorsal and anal fins have not yet completed their development

(Figures 3 and 4). The caudal fin rays are also in the development stage. The notochord tip is not curved (Figures 4 and 5). Therefore, the preflexion larval period continues.

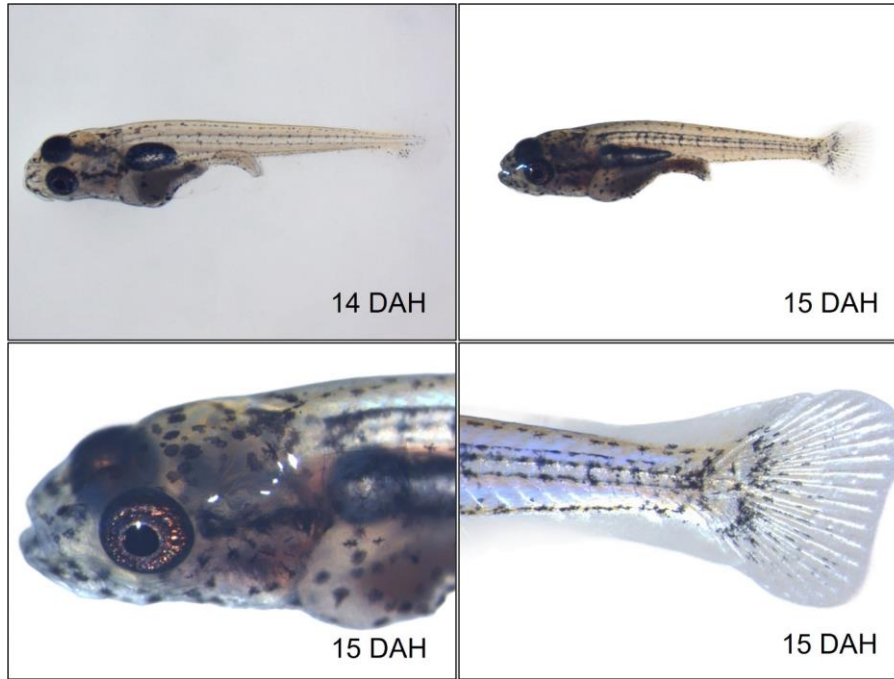


**Figure 4.** Larval development of penguin tetra (*Thayeria boehlkei*). Preflexion larva (8-11 DAH).



**15-16 DAH:** The most important morphological event in these days is the curving of the notochord tip (Figure 5). Therefore, these days can be defined as the flexion larval period for penguin tetra larvae. The formations of the anal and dorsal fins have started to become a little clearer (Figure 5). The

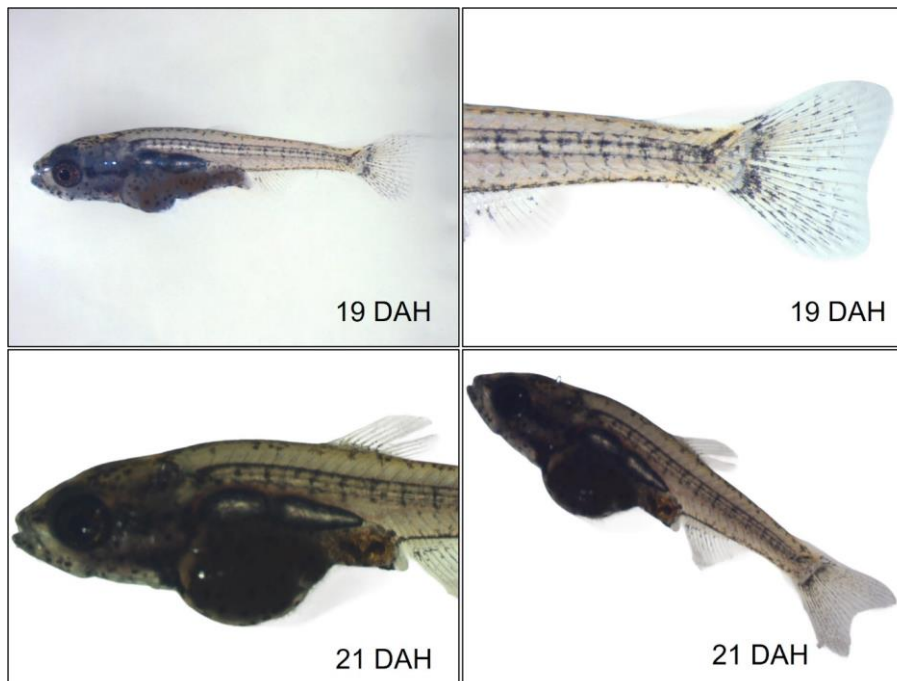
caudal fin rays have also emerged. Body pigmentation has increased compared to previous days. On the 15<sup>th</sup> day, the second chamber of the swim bladder has formed. Thus, the swim bladder has become two-chambered (Figure 5).



**Figure 5.** Larval development of penguin tetra (*Thayeria boehlkei*). Flexion stage, notochord flexion started (15 DAH); flexion stage, the notochord was completely flexed (16 DAH), swim bladder with two chambers was visible (15 DAH).

**19-21 DAH:** The dorsal and anal fins are quite developed, and the caudal fin has taken a forked shape (Figure 6). Body pigmentation has started to enter the color form of the parents

(Figure 6). The transparent appearance of the larva is about to disappear.



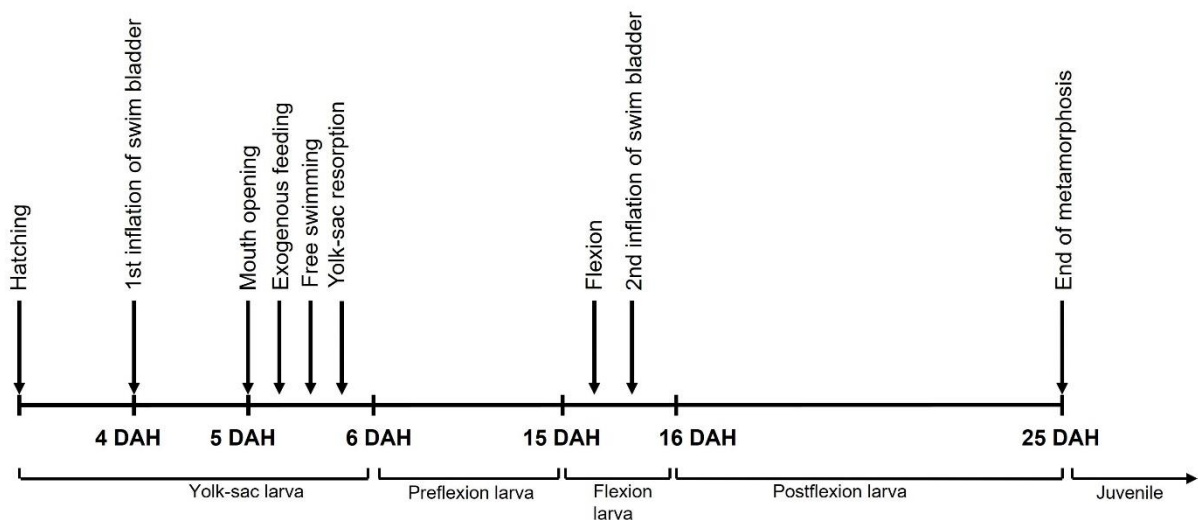
**Figure 6.** Larval development of penguin tetra (*Thayeria boehlkei*). Postflexion larva.

**23 DAH:** All fins have become more shaped and have reached the final stage of their development (Figure 7). The transparent body color of the larva has completely disappeared, and their colors resemble the color of adult individuals. During these days, a black line unique to the penguin tetra species has formed on the side of the body, extending from the head region

to the lower fork tip of the caudal fin (Figure 7). Larval development is morphologically complete (Figure 7). The larvae have now taken on the color and body form specific to the penguin tetra species (Figure 7). Therefore, during these days, the larval stage is completed, and the juvenile stage has been entered.



**Figure 7.** Juvenile of penguin tetra (*Thayeria boehlkei*). End of metamorphosis. The body shape of larvae and pigmentation pattern were similar to those of the adult (25 DAH).



**Figure 8.** The main events of larval development in penguin tetra (*Thayeria boehlkei*).

## 4. Discussion

Tetra fish are small, colorful, and popular freshwater aquarium fish belonging to the Characidae family (Helfman et al., 2009). Understanding the larval development process of these fish from hatching to the juvenile stage can help comprehend the reproductive biology, growth potential, and survival rates of that species (Nakatani et al., 2001).

Tetra eggs are usually transparent, round, and small. The larvae that hatch from these eggs are quite small, usually transparent in color, and underdeveloped. Newly hatched larvae are dependent on their yolk sacs for nutrition until they start external feeding. For tetra larvae to develop and survive, it is critical to provide them with appropriate living conditions and nutritious live foods such as rotifers and *Artemia nauplii* (Çelik & Cirik, 2020; Lipscomb et al., 2020). Therefore, *Artemia* is preferred as the first feed in tetra larval feeding. In this study, *Artemia nauplii* were used for the initial feeding of penguin tetra larvae. The aquaculture practices, water quality parameters, and live food feeding protocol used during the larval rearing process directly affect the timing of morphological events in the developing larvae. For example, when comparing the timing of metamorphosis events during the larval development of black tetra and serpae tetra, it is observed that although these two species have similar morphological development findings, their larval growth rates slightly differ from each other. Looking at these two studies, it was reported that black tetra larvae developed slightly faster than serpae tetra larvae (Çelik & Cirik, 2020; Lipscomb et al., 2020). This situation is the same for penguin tetra larvae as it is for all other tetra species. Environmental conditions and feeding protocols are directly related to the development processes and timing of morphological events in larvae. It is known that larvae of the same species have different growth rates under different conditions. Therefore, new studies on the growth and survival of the species are needed to accurately determine the larval development rates of a species.

In this study, it was observed that the body shape, organs, fins, and body color structure of penguin tetra larvae gradually became more distinct within the first month after hatching. Primarily, the development of the head and eye region was observed, followed by the development of the caudal fin, dorsal, ventral, and anal fins. This situation has also occurred in the same way in some other tetra species (Çelik & Cirik, 2020; Lipscomb et al., 2020). Free swimming started after the initial inflation of the swim bladder.

Pigmentation is one of the most important morphological findings of larval development. These findings are even used in species identification of larvae. Therefore, pigmentation formation and intensification are also mentioned when describing larval development. In this study, the distribution of pigments on the body was also mentioned. Fish larvae initially appear transparent. Later, over time, pigment cells form and

develop in their bodies, and when they reach the juvenile stage, the offspring acquire the color structure of their parents. Pigmentation colors and patterns also differ in different fish species. The same situation applies to different tetra species (Helfman et al., 2009). Pigmentation occurs in species-specific color patterns (Nakatani et al., 2001). As larvae grow and approach the juvenile stage, they become colored similarly to their parents' colors. Therefore, describing the pigmentation process during the larval development of fish is critically important.

Just as pigmentation varies according to species, the duration of larval development also varies depending on the species and environmental conditions. It is known that the development stages of tetra larvae generally take 3-4 weeks (Çelik & Cirik, 2020; Lipscomb et al., 2020). At the end of this period, the larval stage is completed, and the juvenile stage is reached. In this study, the larval development process of the penguin tetra examined was also completed in 3-4 weeks. These offspring have become morphologically identical to their parents. However, it may take much longer for larvae to reach sexual maturity. This also varies from species to species.

Examining the larval development of a fish species of economic importance and documenting the findings are important for scientific research. On the other hand, it is also important for professionals who culture this species to improve their aquaculture practices. Examining the larval development of tetra species is similarly important for scientific research. In addition, it is also beneficial for amateur and professional producers who are engaged in the aquarium hobby and breed tetras. Because accurately describing the larval development process of a fish species can reveal valuable information about the reproductive biology, growth dynamics, and survival capacity of that species (Nakatani et al., 2001). Furthermore, detecting diseases and abnormalities that may occur during larval rearing can contribute to raising healthy products for the aquarium fish industry.

In conclusion, the larval development of the penguin tetra species is critically important for understanding the life cycle of this species and using this information to raise higher quality fish generations. Describing the life cycle of an economically important fish species widely used in the aquarium industry, such as the penguin tetra, from hatching to the juvenile stage is important for the continuity of that species and the sustainability of the aquarium industry. Understanding the larval development process of this species can contribute to raising healthier and higher quality fish in the aquarium fish industry.

## Compliance with Ethical Standards

The study was conducted when ethics committee approval was not required. Both authors possess certificate of handling experimental animals.



## Conflict of Interest

The authors declare that they have no conflict of interest.

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