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EARLY DEVELOPMENT OF THE TETRA CARDINALFISH, PARACHEIRODON AXELRODI

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Abstrak

Cardinal tetra, Paracheirodon axelrodi, merupakan ikan hias yang populer di Indonesia dan mancanegara. Penyumbang ikan hias akuarium di Rio Negro, Amerika Selatan. Komoditas ekspor ikan hias ke Amerika, Jepang dan Singapura. Ikan ini sangat sulit dikembangbiakkan di lingkungan aslinya. Informasi ilmiah tentang reproduksi, perkembangan embrio dan larva masih sangat sedikit. Tujuan penelitian adalah untuk memperoleh informasi tentang reproduksi, perkembangan embrio dan larva cardinal tetra. Informasi ini sangat penting untuk meningkatkan perkembangbiakan ikan cardinal tetra dan konservasi ex situ. Sebanyak 20-50 butir telur digunakan untuk mengamati embrio melalui penetasan dengan mikroskop stereo pada perbesaran 50-100x. Embrio diamati dengan mikroskop stereo sebanyak 20-50 butir telur pada perbesaran 1-2,5x. Fekunditas berkisar antara 115 sampai 550 butir telur. Telur berbentuk bulat, transparan, agak lengket dan tenggelam ke dasar. Diameter telur 0,835-2,202 ±3,7 mm saat menetas dan terhidrasi. Panjang larva saat menetas 2,406-2,703 ±86,96 mm. Kuning telur habis setelah 4-5 hari. Bukaan mulut 0,235-0,366 mm cukup untuk memakan nauplii Artemia sp. Larva mulai berenang bebas setelah 4 hari. Perkembangan larva hingga tahap akhir memakan waktu 28-30 hari.

Kata Kunci: Embrio, larva, perkembangan, pertumbuhan, pembiakan, konservasi

Abstract

The cardinal tetra, Paracheirodon axelrodi, is a popular ornamental fish in Indonesia and abroad. Contributor to ornamental aquarium fish in Rio Negro, South America. Export commodity of ornamental fish to America, Japan and Singapore. This fish is very difficult for breeding in its native environment. There is little scientific information on reproduction, embryonic and larval development. The objective of the research was to obtain information on the reproduction, embryonic and larval development of the cardinal tetra. This information is essential to improve the breeding of cardinal tetra fish and ex situ conservation. A total of 20-50 eggs were used to observe embryos through hatching with a stereo

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microscope for 20-50 eggs at 1-2.5x magnification. Fecundity ranged from 115 to 550 eggs. Eggs are round, transparent, slightly sticky and sink to the bottom. Egg diameter 0.835-2.202 ±3.7 mm at hatching and hydration. Larval length at hatching 2.406-2.703 ±86.96 mm. Yolk depleted after 4-5 days. Mouth opening of 0.235-0.366mm is sufficient for consuming the Artemia sp. nauplii. Larvae start swimming freely after 4 days. Larval development to the final stage takes 28-30 days.

Keywords: Embryo, larva, development, growth, breeding, conservation

INTRODUCTION

Cardinal Tetra (Paracheirodon axelrodi Schultz, 1956) is a key ornamental fish export commodity from Brazil, particularly sourced from the Rio Negro, where it is one of approximately 100 ornamental fish species, contributing to 80% of the local fishermen's catch(Benzaken et al., (2015); Alho et al., (2015)). Despite its popularity, the cardinal tetra faces challenges in being successfully spawned in its natural habitat, a key issue addressed by the Piaba project, which was initiated to conserve these species. In Indonesia, the cardinal tetra was introduced in the 1990s and has since been cultivated by ornamental fish farmers in Bojongsari and Curug, Depok, West Java (Mikkola, 2024). According to KKP (2021), Indonesia's ornamental fish exports in 2019 were valued at IDR 79.9 billion, with cardinal tetras among the exports, reaching 51 destination countries. Major markets for this fish include the United States, Japan, and Singapore, where the price per fish ranges from USD 1.00 to 2.99, and can go as high as USD 5.49 in international online stores. Locally, the price of cardinal tetras ranges from IDR 700 to 3000 depending on size.

Scientific studies on reproduction, embryos and larvae in cardinal tetras are few. Previous research on this fish includes oogenesis development, fish transportation, feeding spectrum in nature, growth and survival, molecular and tolerance to water quality (Yen, (2013); Gomes et al., (2009); Nurhidayat et al., (2016); Moller & Beheregaray (2004); Mirande, (2019); Oliviera et al., (2008)). Ex-situ conservation is an approach used to preserve endangered species by maintaining and protecting these organisms outside their natural habitat. This method involves efforts to build healthy populations in controlled environments, such as zoos, conservation centers, or breeding facilities (Moloney et al., 2023). Ex-situ conservation plays a crucial role, especially when threats to species in the wild are significant, such as habitat loss, climate change, or illegal hunting. By preserving species

outside their natural habitats, we can ensure their long-term survival while seeking solutions to restore their habitats in the wild (Cabrita et al., 2022).

The successful breeding of Cardinal Tetras outside their natural habitat can make a significant contribution to the conservation of this species (Rincón-Camacho et al., 2022). By creating well-breeding populations in ex-situ facilities, the pressure on wild populations can be reduced. Successful breeding allows for the distribution of healthy individuals back to their natural habitats or even facilitates more sustainable trade, reducing direct exploitation of wild populations (Walster, 2024). Additionally, a better understanding of the life cycle and larval development of Cardinal Tetras can support efforts to restore their populations in the wild, reducing reliance on wild capture and helping to conserve their ecosystems (Goodall et al., 2024).

The spawning success of the Cardinal Tetra (Paracheirodon axelrodi) in its natural habitat is limited, as these fish cannot yet be naturally spawned outside of their native ecosystems, such as in the waters of the Rio Negro (Cavallino et al., 2023). Despite efforts to breed them in artificial environments, existing methods, such as spawning in aquariums with controlled temperature and water quality settings, face major challenges in accurately replicating their natural conditions. One of the main challenges is the lack of understanding of the life cycle of these fish, particularly at the embryonic and larval stages (Imtihani & Nasser, 2024). The aim of this research was to evaluate embryonic and larval development in cardinal tetra. The research conducted can provide important information such as the timing of larval hatching, when larvae start feeding, feeding according to mouth opening, growth stages and timing of fingerlings. This is expected to improve the quality and quantity of cardinal tetra production. The success of cultivation outside its natural habitat also supports the conservation of cardinal tetras ex-situ.

RESEARCH METHODS

A completely randomized design is an experimental method in which experimental units (e.g., individuals or groups of larvae) are randomly divided into different treatments or groups without considering other factors that might affect the results (Khaira et al., 2024). The tools used were 80×40×20 cm3 aquarium, 20×10×5 cm3 aquarium, Taffware® portable microscope, Olympus® SZX-10 stereo microscope, Olympus® embedded computer, Kandila® aquarium led lamp, plastic pipette, Shinpo® plastic container, Onemed® petri dish, Terumo® 1mL syringe with needle, Materials used in the study were test fish in the form of tetra cardinal fish cultivated by ornamental fish farmers in Curug, Bojongsari,

Phenoxyethanol and 10% formalin. Spawning eggs are collected in a quantity of 20-40 larva and placed in plastic containers of 15 x 15 cm2. Observation under a microscope is done using a petri dish with a diameter of 5 cm and a little water with the water level half the height of the petri dish. Egg position is adjusted using a needle or tweezers as needed (Jalan Perikanan Nomor & Kampung Baru, n.d).

Larval observations were made by placing 20-30 larvae in 15 x 15 cm^2 aquarium containers. Observations are made every 12 hours to monitor larval development. The temperature of the larval rearing medium should be within a more realistic range of 25-28°C, which is the ideal temperature range for fish larval development. Room temperature ranging from 26-27°C supports temperature stability in the aquarium container, but it is important to ensure that the water temperature remains within the optimal range to prevent stress to the larvae (Jalan Perikanan Nomor & Kampung Baru, n.d). Apart from temperature, water quality is also an important factor in larval rearing. Water quality parameters that should be monitored include pH, which should ideally be in the range of 6.5-7.5, as well as dissolved oxygen levels that need to be kept sufficient to support larval respiration. A good oxygen level should be above 5 mg/L. To maintain water quality, aeration systems are often used to ensure dissolved oxygen remains optimal.

In addition, filtration can also be applied to remove impurities or organic matter that can damage water quality. The parameters measured were larval length (TL and SL), yolk absorption rate, and larval mouth opening width. Observations of embryos and larvae were performed under an Olympus® brand stereomicroscope, type SZX10, equipped with an Olympus® DP21 digital camera connected to an Olympus® embedded PC. Data collected from each stage of embryonic and larval development to definitive stage are descriptively examined for each developmental period in accordance with (Wijaya, 2024).

RESULTS AND DISCUSSION

Embryonic Development

The eggs were collected at 7:00 a.m. and the eggs had reached the stage of embryo emergence in the meridian. This stage marks the end of gastrulation with 100% closure of the epiboly (Permana et al., 2020). In Hypessobrycon pulchiprinnis, development reaches the final gastrulation phase at 180-240 minutes after fertilization. Based on (Cole & Haring, 1999) the estimated spawning time of this fish is 03.00-04.00 a.m. early in the morning.

Like the eggs of rasbora (Rasbora argyrotaenia) and featherfin catfish (Synodontis eupterus), which are transparent and still translucent. Cardinal

tetra eggs are round, not sticky and sink to the bottom of the tank. Egg diameter ranges from 0.835-2.202 ±3.7 mm with a fertility of 126-550 eggs. The perivitelline space forms between the chorion and the yolk shortly after fertilization. The embryo begins to take shape at 4-5 hours after spawning, with the head and tail already distinguishable. By hour 9, the tail has begun to elongate and separate from the yolk. The eyes begin to appear at 12 hours, followed by the tail turning to the right or left of the yolk. The stages of embryonic development are shown in Table 1.

1. Cleavage Phase

The cleavage phase begins when the blastodisk has formed and divides into two to about 32-64 cells. In the cleavage phase, the blastodisk divides according to the cleavage plane and divides into two with the same shape, the process continues onwards (Cole & Haring, 1999). This phase is not well observed and development has entered the stage of gastrulation and embryo shielding which is characterized by closing the blastomeres by 50%. Whereas in neon tetra fish, Paracheirodon innesi, the 1-2 cells division phase lasts for 11 minutes and the phase up to 128 cells is 2 hours 18 minutes after fertilization (Lythgoe & Shand, 1983).

Table 1. Embryonic Developmental Stage of Tetra Cardinal Fish (*P. axelrodi*).

No	Phase	Time		Stages of Development
		Hour	Minute	
	Cleavage Stages			
1	Division 1 -64 cell	0	2	Approx from Latifah, 2023
	Blastula Stages			
2	Division 256 cell up	2	50	Approx from Latifah, 2023
	Gastrula and Embryonic Shield Stages			
9	Embryonic shield (Epiboly 50%)	3	31	Blastomere covered 50% yolk
10	Epiboly 60%	4	10	Blastomere covered 60% yolk
11	Epiboly 90-100%	4	58	Blastomere covered 90-100% yolk
	Segmentation and Organogenesis			
12	Embryo becoming visible	5	06	Embryo visible in meridian
13	Embryo developing	6	16	Segmentation and organ formation
14	Late embryo	18	50	Embryo becomes very active
15	Hatching	20	04	Embryo hatches to larva

2. Gastrulation Phase and Embryo Shield

The results showed that the phase of cleavage to 50% epiboli lasted for 3 hours 31 minutes and 90-100% epiboli occurred 4-5 hours after

fertilization. Lythgoe & Shand, (1983) stated that in the neon tetra, Paracheirodon innesi, this phase occurs at 5-6 hours after fertilization at a temperature of 25 ± 2.0 oC. Woynarovich & Horvath, (1980) state that temperature affects embryo development and larval hatching rate. If the temperature is high, the larvae will hatch prematurely, whereas if the temperature is low, the larvae will hatch late or be retained in the chorion. The embryonic development and hatching larvae are shown in Figure 1 A-E.

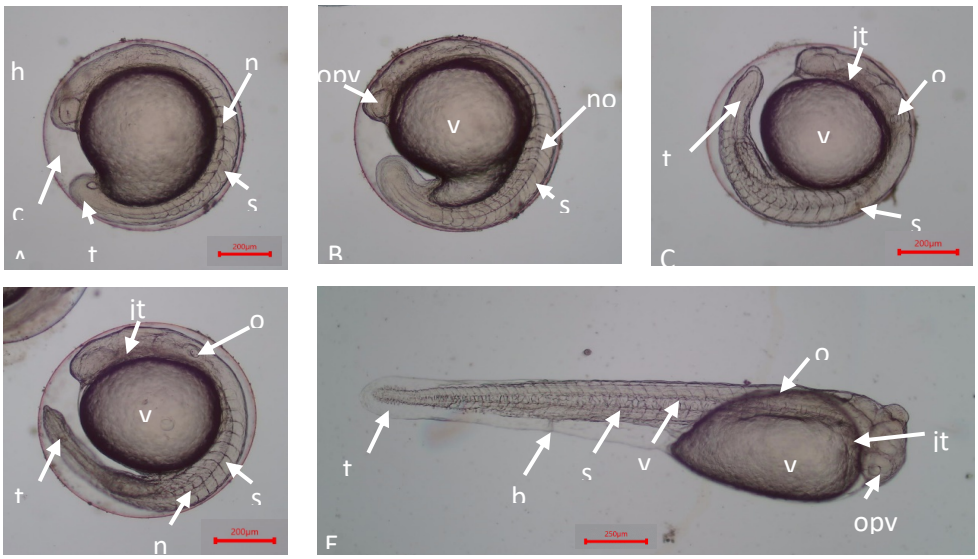


Figure 1. A. Embryos at 4-5 hours post-fertilization, with the following structures visible. B. The embryo at 6-7 hours after fertilization, and C. The tail of the embryo begins to elongate, forming spinal tissue, tail (t), and notochord (no). D. The otic vesicle commences its development into the otolith (ot). E. Following a period of 18-20 hours during which the larva will proceed to hatch, with the head or tail emerging first. head (hb), yolk (y), eye (opv), chorion (ch), tail (tb), somites (so), and spinal axis (no). anal opening bud (ba), vertebrae (vt), and otoliths (ot). (nt), heart (jt).

3. Segmentation and Hatching

The appearance of the embryo at 4-5 hours post-fertilization is characterized by the formation of the head bud at the animal pole and the tail bud at the vegetal pole. Additionally, the epiboli have reached their maximum closure by this stage. During this period, the cells undergo a series of changes in shape, forming the eyes, notochord, heart, and other organs in accordance with their respective functions. At the 11th hour, the eye is formed, which is characterized by the presence of an optic vesicle, or eye socket, followed by the formation of a notochord, or spinal axis. In

zebrafish (Brachydanio rerio), wild betta fish (Betta imbellis), and Papuan rainbow fish (Melanotaenia sp.), eye development is characterized by the onset of pigmentation in the eye, which is often referred to as the eye spot phase. This pigmentation occurs while the larvae are still within the chorion. In contrast, in cardinal and neon tetras, pigmentation occurs subsequent to larval hatching.

The otic vesicle is already visible in the embryonic phase and is located in a posterior position, in a bubble-like shape with two dots. During its development, the otic vesicle moves cranially, while the otolith moves caudally (Gochee & Staecker (2013); Kursini et al., (2015)). At the late embryonic phase, which occurs at 20 hours, the embryo displays extensive organ development within the chorion and a functioning heart. As the hatching phase is approached, the movement of the embryo accelerates. Embryonic movement is a mechanism that occurs during the hatching process, with the objective of breaking the chorion. Braum (2000), posits that hatching occurs as a result of the embryo's vigorous movement, coinciding with the softening of the chorion layer. In addition to the aforementioned movement, the softening of the chorion is also facilitated by the activity of enzymes. The larval stage of the cardinal tetra fish is completed after 18-20 hours of development. The larvae typically emerge from the egg in a head- or tail-first orientation. In contrast to P. innesi, the hatching process occurs over a longer period of time, with the larvae emerging 24 hours after fertilization.

Larval Development

The larval development process is divided into four main stages: 1. Viteline Larvae: Duration ranges from 1 to 4 days post-hatching (DAH). This phase emphasizes survival, with the larvae depending on yolk reserves. Eye pigmentation initiates, and the digestive tract commences formation. 2. Pre-flexion Stage: From 4 to 8 DAH, this phase signifies the shift to external nutrition when the yolk is assimilated. 3. Flexion Stage: Transpires approximately 11-13 days after hatching, marked by the bending of the notochord and the formation of the caudal fin. 4. Post-flexion Stage: Spanning from 13 to 30 DAH, this final stage involves fin ray segmentation and scale emergence, completing in development by approximately 22 days post-hatching (Stevanato & Ostrensky, 2018).

1. **Viteline Larvae Stage.**
   The larvae emerge from their eggs after a period of 19-20 hours at a temperature of 25-26°C. During this phase, the larvae are not yet capable of swimming and exhibit only limited movement at the bottom of the aquarium. The length of the larvae ranges from 2.406 to 2.703 ±86.96

mm. Cardinal tetra larvae are equipped with an attachment device on the dorsal surface of the head, enabling them to adhere to the substrate (Benedito-Cecilio & Araujo-Lina, 2002).

2. **Pre-flexion Stage**

At this stage, yolk sac absorption is observed (Chart. 1). At the 12th hour, the yolk is absorbed by 20%, and at the 24th hour, it is absorbed by 35%. Absorption begins to increase at the 48th hour, achieving a rate of 83.3%, and thereafter decelerates until the 96th hour, completing at 100%.

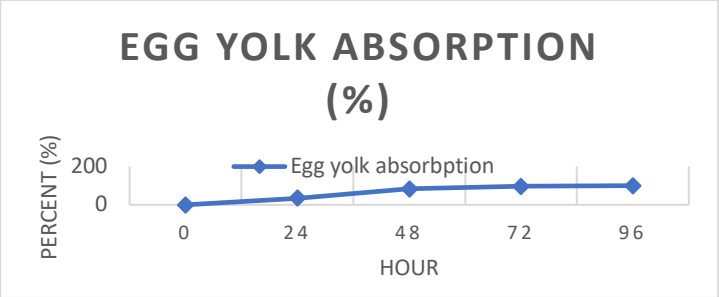


Chart 1. Egg yolk absorption percentage during larva development, from 0-48 hours fast absorption then slow absorbing untill 100%.

On the 4th and 5th day of larval life, the inflation of the swim bladder begins, when the larval mortality rate is highest (40-50%) and the consumption of exogenous food begins, with active pursuit of small prey. The larvae length ranges from 3,78-4,42 mm, eyes with pronounced metallic blue pigmentation and a partially pigmented body. especially in the head region. During this period, the pectoral fins are fully formed. As the yolk is depleted, the swim bubble enlarges.

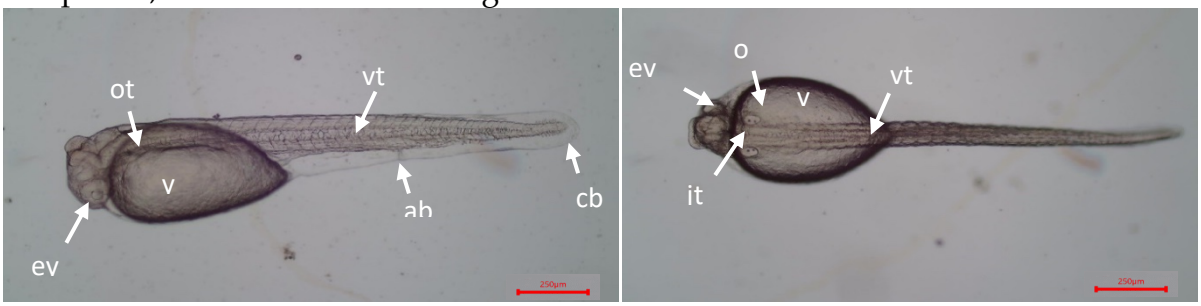


Figure 2. A. Newly hatched of cardinal tetra larva (ventral view). B. Dorsal view of newly hatched larvae. Eyes (ey), yolk egg (y), anal bud (ab), otolith (ot), heart (jt), vertebrae (vt), caudal fin bud (cb).

The larval growth of cardinal tetras can be considered slow compared to other teleost fish species, especially rheophilic ones such as matrinxã (Brycon cephalus), which takes the same shape as the adult 48 hours after fertilization (Nurhidayat et al., 2015). In Brycon insignis,

individuals have the same shape as adults 94 hours after fertilization, when they reach a total length of about 1.2 ± 0.24 cm. The formation of fin organs is a crucial aspect of larval development in certain fish species. In P. axelrodi fish, fin development progresses sequentially from the pectoral, caudal, ventral, anal, to the dorsal fins. In these stages, there are no notable alterations in the morphology and development of the fish's organs. The maturation of larvae significantly enhances the stages of organogenesis, including; The elongation of anal, caudal, and dorsal fins is increasing. The larvae's total length expands to 6,087–6.448 mm, and black pigments or bars on the head and body start to manifest

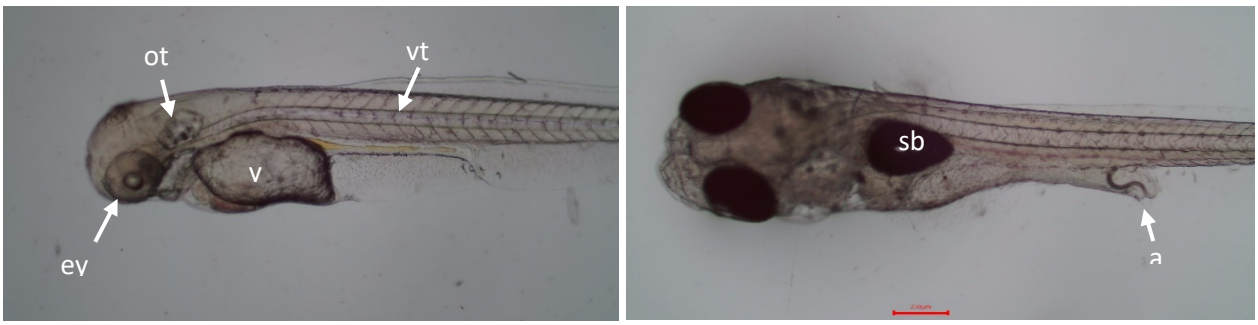
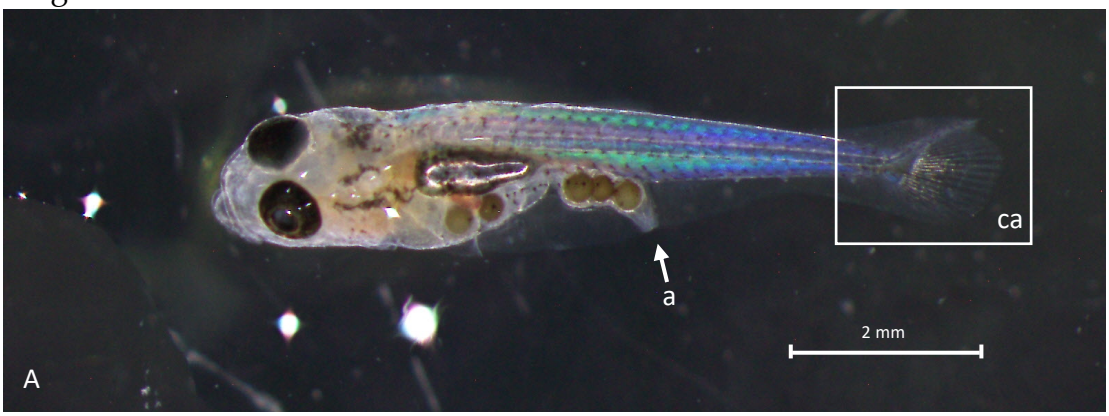
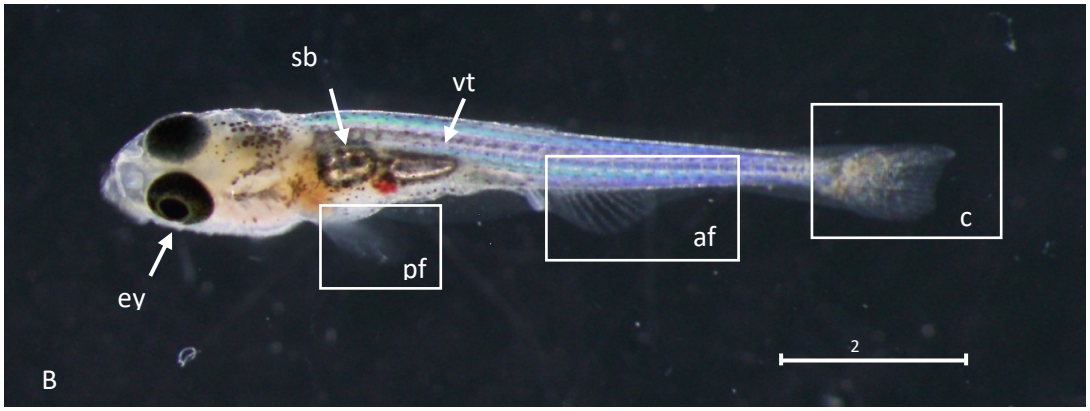


Figure 3. A. Larva of cardinal tetra 48 hours after hatching. B. 5 days or 120 hours after hatching. Description: eyes (ey), otolith (ot), vertebrae, yolk (y), swim bladder (sb) and anus (a).

3. Flexion Stage

On day 12 of life, the first red pigment is visible at the base of the anal fin and soon after, in the ventral region, soon after in the ventral region and caudal peduncle (Figure 4A). The metallic blue pigmentation, until then confined to the eyes, begins a slow process of expansion to the base of the pelvic fin. The larvae at this stage range between 6.64 mm and 7.50 mm in length. Picture of

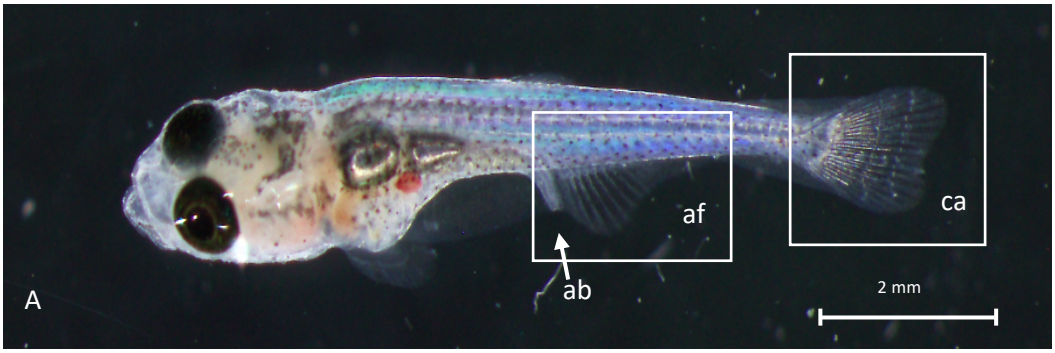


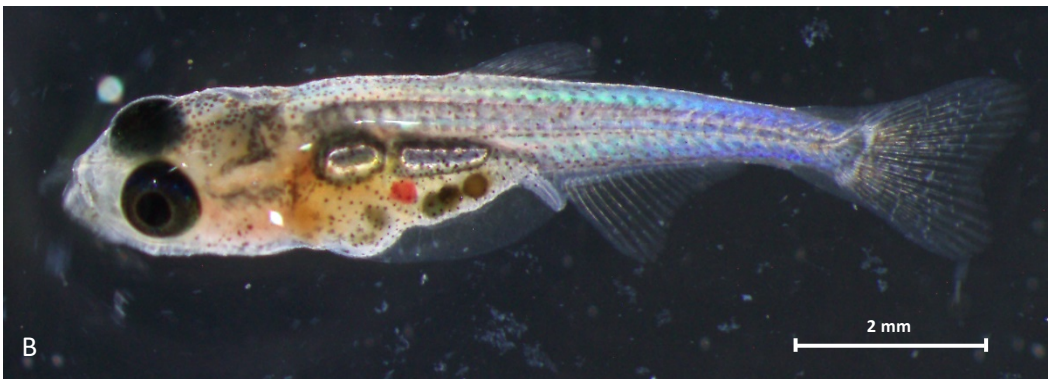


Figures 4. A. Larva of cardinal tetra 12 days after hatching. B. 14 days or 336 hours after hatching. Description: eyes (ey), otolith (ot), vertebrae, yolk (y), pectoral fin (pf), anal fin (af) and anus (a).

4. Post-flexion Stage

At the age of 14 days after spawning (Figure 4B), the tip of the tail begins to curve upwards, which marks the formation of the tail. The caudal fin of the cardinal tetra is a fork (lightly) or "cagak" type with two upper and lower ends.





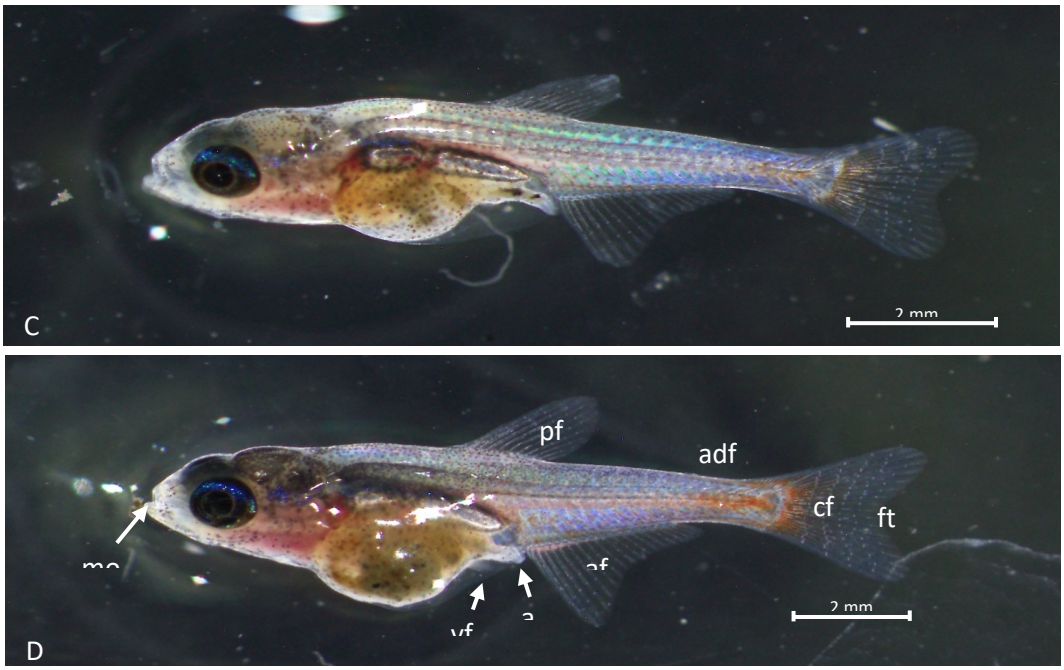
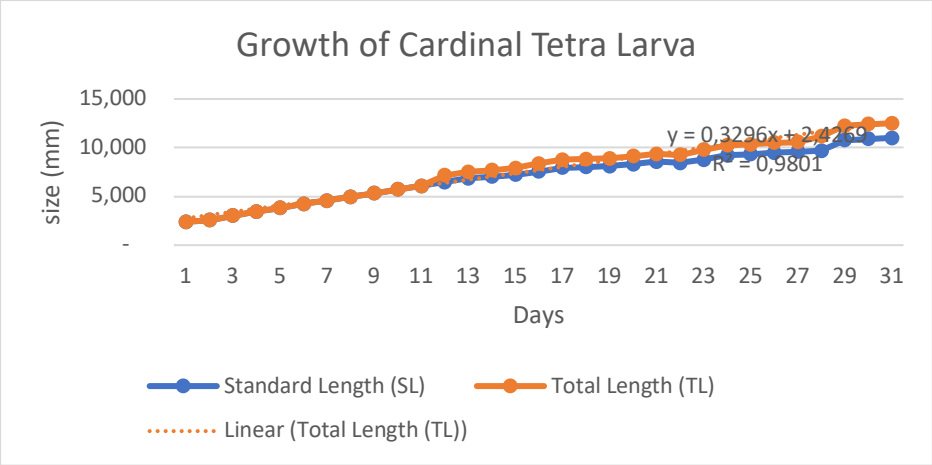


Figure 5. Larval development of P. axelrodi at 14 days (A), 20 days (B), 25 days and 28-30 days post-hatching. Fully grown larva that looked like the adult (D); mouth opening (mo), pectoral fin (pf), ventral fin (vf), anal fin (af), adipose fin (af), caudal fin (cf), forked type caudal fin (ft).

On day 20, the tail has formed a forked tail with fin rays inside. The adipose, dorsal, ventral and pectoral fins also grow a lot. The larvae's length in this stage ranges from 9.35 to 9.70 mm. On day 25-28 all fins possess class radii and are not amalgamated with the fin membrane and all pigments on the head Figure 5B to 5C. Length of larvae ranges from 11.17-12.39. The prospective brood (Figure 5C) is confirmed to possess the same morphology as the larvae at 30 days of age is the concluding period of larval development, wherein the larva attains a form that is definitive or equivalent to that of the adult. The developmental process of P. axelrodi larvae completes between the 28th and 30th day after-hatching and body are identical to those of adults.

Chart 2. Growth of Cardinal Tetra Larva for 30 Days



The total length of neon tetra fish larvae is progressively increasing daily, with standard length growth paralleling the total length increase. Total length refers to the measurement of the fish from the anterior tip of its mouth to the posterior end of the caudal fin (TL). The standard length is defined as the measurement from the anterior end of the mouth to the posterior end of the vertebrae (SL). Graph 2 illustrates the growth of cardinal tetra fish over a 30-day period, demonstrating positive growth characterized by a linear curve and a R value of 0.9801. The growth from hatching to day two exhibits a straight pattern, becoming fluctuating afterward. The preliminary development of the tail fin commenced between day 12 and day 14, during which the tip of the tail began to elevate and the predecessors of the fin rays underneath started to form. Between days 28 and 29, growth surged significantly before thereafter stagnating once more.

CONCLUSION

Fecundity of the cardinal tetra P. axelrodi ranges from 115-550 eggs. Eggs are sinkable, non-sticky and transparent. Embryo development occurs for 18-20 hours and hatches at hour 20. The yolk is absorbed on day 5-6. The mouth opening on day 4 is 0.235-0.366mm and can already be given Artemia nauplii (0.315-0.386mm). Larval development lasts for 28-30 days until it becomes definitive, and to reach S size (1 cm TL), 100-120mm, it takes about 38-40 days. Production of this fish has become relatively stable each year with breeding in Indonesia and Vietnam, reducing dependence on the supply of ornamental fish, especially wild-caught cardinal tetras.

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