Microscopic Larvae Stages of Kurumoi Rainbow Fish (*Melanotaenia parva*) First to Eight Days

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Abstract. Kurumoi Rainbow is one of an endemic fish from Kurumoi Lake, West Papua. The purpose of this study was to observe the development of *M. parva* microscopically from the first day to the larvae of the eight day. Measurable data are: total length of larvae; the size of the mouth opening of the larvae; percentage of egg yolks; and microscopic photos of larvae taken using Olympus type SZX9 microscope. Results of measurements respectively for larval length (mm); size of mouth opening of larvae (mm), percentage of yolk (%) as follows: D0 / first day (4.61, 0.052, 100), D1 / second day (4.90, 0.069, 20.7), D2 / third day (5.01, 0.095, 13.4), D3 / the fourth day (5.42, 0.105, 0), D4 / the fifth day (5.64, 0.16 0, 0), D5 / the sixth day (5.84, 0.248, 0) and D7 / the eight day (7.789; 0.251, 0). The total length of the larval body and the size of the mouth opening increases when an age increased. The size of the mouth opening is to measured the type of natural feed so as to optimize the survival of the larvae.

1. Introduction

Rainbow fish is one of the ornamental fish that has uniqueness on the color of its scales varies and resembles a rainbow. Kurumoi rainbow fish is one of Indonesia's original freshwater ornamental fish which has a beautiful color like a rainbow so it has aesthetic value and value economically high [1]. This fish is an endemic to Lake Kurumoi, Papua [2]. Rainbow fish is a species of aquatic fish the largest bargain in the waters of the Continent Australia and Papua Island [3]. Believed to South Sorong as a place of germplasm center species of rainbow fish *Melanotaenia* spp. [4]. This fish entered the Melanotaniidae family of one the species is kurumoi rainbow fish (*Melanotaenia parva*). According to Tappin [3], there are 95 species of rainbow comes from Sulawesi and Papua. This fish interesting because of the whole male sex orange body.

According to [2] based on observations in 2007 conditions Lake Kurumoi has been increasingly narrowed due to erosion heaps and lack of river water discharge, less than 1 hectare of lake still remaining water. The condition of the lake becomes shallow with water height less than 1 m and conditions in the lake there are tilapia fish (*Oreochromis mossambicus*) are abundant. Kurumoi rainbow fish becomes very
reduced and difficult to captured, these fish can now be found and captured in the narrow creeks resulting from the presence of erosion in the dry part of the lake and pressed by the population of tilapia.

Freshwater ornamental fish business and trading is quite good role in providing foreign exchange to the state, however when compared with the needs of ornamental fishworld, its value is still relatively small [5]. On the other hand, conservation of rainbow fish from extinction needs to be done immediately on a regular basis insitu or exsitu. Exsitu, conservation can be done by doing the fish development activities outside. Publications on the development of embryos and larvae from rainbow fish are still rare [6]. Kurumoi rainbow fish (*Melanotaenia parva*) has already been domesticated and spawned in Research Institute for Ornamental Fish Culture (BRBIH) Depok, West Java, Indonesia. During the cultivation and mass production the survival of larvae almost low. To increase the larval constraints especially in the early critical period of larvae, is necessary conducted observation activities of larval development. The embryonic development mechanism of teleostei fish is essentially the same, which is distinguishing is the length of time the process of development. This process is controlled by genetic factors and influenced by environmental factors. Factors that directly affect the speed embryo development is a striking variation in egg size between species and incubation temperature [7].

These observations include activity absorption of egg yolks as endogenous nutrients, size of mouth opening, and completeness fins in the larval stadia. This study aims to determine development of morphology of rainbow fish larvae kurumoi and is expected to be a reference for appropriate natural feeding management for supporting the success of the cultivation of rainbow.

2. **Experimental**

The materials used in this research were: the broodstock of kurumoi rainbow fish consisting of 4 male (5.0-15.4 g; 6.3-10.6 cm) and 5 female (7.1-12.7 g; 7.5-9.7 cm). Fertilized eggs, freshly hatched larvae as a larvae named D0, frozen bloodworm as broodstock feeds and natural feed for larvae.

Equipment were used during the research, among others, namely: concrete tank for broodstock spawned in the form of a size of 2 x 1.5 x 0.7 m which was equipped with a tub filtration and recirculation system. In addition there was an egg hatching container in the form of a plastic basin with a volume of 5 liters of water. Raffia rope as a shelter that is made in such a way resembling an aquatic plants was needed to attached all the eggs produced. Aerators, hoses, sesers or drains, digital scales, millimeters of blocks and daily tools were used for fish maintenance. for the purposes of larval surveillance, microscopes equipped with micrometers and cameras were connected to the computer (Olympus type SZZX9 microscope ) and small petridisks for larval observation containers were required.

2.1 **Spawning of fish, egg hatching, feed larvae**

Spawning was done in the concrete tank dimension 2 x 1.5 x 0.7 m with 46 cm high water with recirculation system. As the paste medium was used raffia straps that have been parsed and weighted bottom part. Spawned eggs had attached to the rope, eggs which had fertilized then placed on a plastic basin container for incubation. Incubation was performed for ± six days (at temperature 26°C-27°C). After the eggs hatched, larvae kept in aquarium size 50 cm x 50 cm x 40 cm with water height 15 cm. On this aquarium maintenance was aerated with smooth bubbles to supply oxygen. Water temperature conditions in aquarium was maintained during observations ranging from 27°C- 28°C. Siphon basic aquarium maintenance was done every three days. Feed management on larvae was the adaptation of natural feeding started on the second day after hatch where there was still a small portion of egg yolks but the larvae had been fed with the purpose of feed adaptation. Given the infusoria's natural feed that swims in the water would stimulate the larvae to pursue infusoria and open the mouth as a stimulus of physical consuming movement. So that when the yolk was completely exhausted, the larvae did not experience shocked due to changes in endogenous feed to exogenous feed.
2.2. Observation of larval morphology

Observation of larval development on this study was conducted for 8 days. Observations began at the time of the new larvae hatch (D0). The larval samples were taken by use plastic pipes 3 mL and then placed on the small petridisks to observed under a microscope. Number of samples at each the observation time was three larvae. The parameters observed were total length, mouth opening, yolk egg’s volume, yolk egg’s absorption, and the development of organogenesis during 8 days. Observation parameters of total length, mouth opening, and the development of organogenesis were observed every day for 8 days, while parameter observation of yolk egg’s volume and yolk egg’s absorption observed during 60 hours after the larvae hatch at intervals observation every 12 hours.

3. Results

3.1 Daily increase of larval length

The total length of rainbow kurumoi larvae at the beginning of the hatch is about 4,614 mm ± 0,049. This size is almost the same with another Melanotaenian genus, ie measuring 3.4-4.2 mm [8-9].

![Figure 1: Growth performance in total length of kurumoi’s rainbowfish larvae](image)

Growth performance during this research is shown in Fig.1. Based on Fig.1 it can be seen that growth larval rainbow kurumoi during 7 days increases with increasingly age of fish. After hatch to the sixth day of total length is relatively slow but can be seen on the seventh day of growth rising rapidly to reach a total length of 7,789 mm ± 0,001. According to [6], growth of Melanotaenia splendida fish larvae splendida (Peters) also runs slow with a relatively small variation in the first 12 days after the larvae hatch. Pramono [10] reported that the total length of larvae fish Brek looks increasing along with age. The process of adding length Brek fish larvae total from day to day also utilize the yolk as a source of energy. On when the egg yolk has been absorbed, the total length of larvae reaches 6.52 ± 0.31 mm.
3.2. Mouth opening of the larvae

Movement of openings the mouth of the curlyfish rainbow larva begins with the movement of the lower jaw slowly then followed by movement of his upper jaw until the end both jaws are equally moving, but from first hatching up to the first 12 hours larvae rarely open their mouths.

![Figure 2](image)

**Figure 2.** Mouth size opening of kurumoi’s rainbowfish larvae

The mouth of the kurumoi rainbow larva has gone develop and start to open for a moment after hatching with the size of the mouth opening initial 0.052 mm ± 0.010. During age 0-3 days of mouth opening of larvae ranged between 0.052-0.105 mm. Hence, find the appropriate size and type of natural feed with the size of the mouth opening for the larvae support successful handling on the first critical period. Type of food can be given is infusoria (0.090-0.110 mm). According to [6] the larvae of the rainbow fish begin to eat a few hours after the larvae hatch.

Size of mouth opening at larvae age 4-7 days ranged between 0.160-0.251 mm; type of food which matches the size of the mouth opening of the larvae at this age is from the Rotifera class (0.090-0.300 mm) [11].

3.3 Yolk egg’s volume

Based on Fig.3, 12 hours after the eggs hatch, the yolk volume has decreased considerably, from 0.1870 mm$^3$ to 0.0851 mm$^3$ and then at 60 hours after the yolk hatch is almost completely depleted in 0.0185 mm$^3$. A critical period in the larvae is the transition period of the endogenous energy (yolk egg) to exogenous energy (zooplankton) referred to [12].
Activity yolk egg absorption of rainbow larvae kurumoi takes place very quickly start from the time of hatching up to the first 24 hours. At the first 24 hours after hatching, egg yolk has been absorbed by 79.3%. At 48 hours the egg yolk has been absorbed up 86.63% (Fig.4). Volume of yolks egg larvae kurumoi rainbow fish began totally to be absorbed on 60 hours after hatching. [13] said that availability the type of natural feed that fits the size the mouth opening of the larvae in sufficient quantities within the period of time is the absolute requirement that must be fulfilled to ensure the subsequent larval conformity. Inadequate nutrition given can inhibit the process of formation and growth of larvae, even can cause death of larvae.

3.4. Yolk egg’s absorption
3.5 Organogenesis D0-D7
Observation of larval development on this study was conducted for 8 days. Observations begin at the time of the new larvae hatch (D0) until D7.

![Figure 5. Organogenesis in rainbow kurumoi larvae aged 0-7 days](image)

Photo source: private collection

Larvae D0. Full-volume yolk egg is located at the bottom of the head with oil granules that are clearly collected in the ventral yolk, gastrointestinal or intestinal tract is completely invisible, the heartbeat is clearly visible, fin candidates have been formed including the caudal fin but the shape is not perfect, eye organ already looks solid black, caudal and dorsal fin fused and pectoral fin only already formed. Different with the fish ‘Corydoras panda’ where the fish does not have oil granules in eggs or yolks egg [14].

- Larvae D1. The volume of the egg yolk is much reduced, which means egg absorption, the edge of the eye is more pronounced, all fins are perfect and C-shaped caudal fins, black spots all over the body surface thinned. Adaptation to exogenous feed started at this stage by giving natural food in the form of infusoria. Egg yolk is composed of lipoproteins which are particles composed of lipids and protein that enables the transport of lipids through the bloodstream. A lipoprotein particle consists over the outer layer of phospholipids, which makes them water soluble. Phospholipids very useful for the early development of fish larvae, both freshwater fish and sea water [15] and also reported by [16] that the development of nutritional source organs is supplied from yellow egg.

- Larvae D2. Egg yolk is almost absorbed all, left very little and almost runs out, begin to look 'intestinal candidate' which is a straight channel, the black corneal border is more clear and contrast with the clear surroundings eye.

- Larvae D3. Intestine contains natural food and begin to appear there is a groove of the intestine, the anus also began to appear, and can be seen the larvae are removing impurities through their anus.

- Larvae D4. Pectoral fins dangle long, black spots thickened and centered along the linea lateralis, the intestines look more clear, blood circulation is also evident from this stage.

- Larvae D5. The skull is clearly visible.

- Larvae D6. Gill organs begin to form, gill closing lines began to appear.
• Larvae D7. The yellow pigment begins to appear in the upper head and across the edges of the body surface, the skull and operculum are perfectly formed, the fingers on the caudal fins begin to have bones, at this stage the larvae are completely mature.

4. Conclusion
The total length of rainbow kurumoi larvae at the beginning of the hatch is about 4,614 mm ± 0.049. Incubation period of their eggs is approximately 6 days. Two-day age larvae have begun to be given natural feed of infusoria as a form of feed adaptation. Infusoria is a type of natural diet suitable for larvae aged 2 days to 8 days and after that can be continued by giving rotifer. Feed management applied in this study, the survival of the larvae increases and can pass the first critical phase in the early stage of larvae.

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