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Research Article
Embryonic and Larvae of Endemic Celebes Rainbow Fish *Marosatherina ladigesi* (C.G.E.Ahl, 1936) (Atheriniformes: Telmatherinidae)

[1]Jayadi, [1]St. Hadijah, [1]Harlina, [1]Rustam and [2]Nursahran

[1]Department of Aquaculture, Faculty of Fishery and Marine Science, Indonesia Muslim University, Jl. UripSumoharjo Km .05 90231 Makassar, South Sulawesi, Indonesia
[2]Departemen of Aquaculture, College of Marine Sciences and Fisheries, BALIKDIWA, Makassar, Indonesia,
Jl. UripSumoharjo Km 11 90231 Makassar, Indonesia

Abstract

Background and Objective: *Marosatherina ladigesi* is locally called the "beseng-beseng' including endemic freshwater fish in South Sulawesi, Indonesia. The objective of this was to describe for the first time the development from eggs to the post flexion larval stage under controlled conditions and was to determine the developmental stages of embryonic and larvae of *M. ladigesi.* This consideration is possible to conduct breeding program of this species commercially and is suitable for commercial culture. **Material and Methods:** Brook stocks were reared for 5 months with fed twice daily with mixture of *Daphnia* sp., *Chironomus* sp. and *Culex* sp. Water was substituted up to 50% each week. Artificial substrate from plastic rope was attached in the tank. The eggs were collected 6 h after laying on the substrate. The larvae were fed twice daily with *infusoria*, *Daphnia* sp. and *Artemiasalina* nauplii. Observations of embryonic development were performed every 6 h until the eggs hatched. Egg samples were observed with 5 eggs at a time. Larvae were observed at the ages of 0.3, 5, 7, 10, 15, 20, 25 and 30 DAH. **Results:** The process of embryonic development takes 204 h after spawning (HAS) or 8.5 days after hatching (DAH). The cleavage stage is composed of 4-8 cell divisions that occur before 12 HAS, 16 cells formed at 12 HAS and 32-64 cells formed at 24-30 HAS, cell multiplication occurs after 36 HAS. The morula stage is 42 HAS, the blastula stage occurs at 54-60 HAS, the gastrula stage occurs at 72 HAS, the neurula stage begins at 84 HAS and the segmentation stage occurs at 96 HAS. The segmentation stage shows already formed eye spots. The yolk stage lasted until 5 DAH, the free flexion stage occurred at 7 DAH, the flexion phase at 10 DAH and the post flexion stage at 15 DAH. The mouth was open65-78 HAS or 3 DAH and the yolk reserves were used for 5 DAH. **Conclusion:** *M. ladigesi* eggs are very adhesive and do not float and the eggs hatch to larvae at 204 HAS or 8.5 DAH at 29±1°C. This study revealed that the flexion stage occurred after 10 DAH and the post flexion stage after 15 DAH. The mouth was open after 65-78 h (3 DAH) and the yolk sac reserves were used for 5 DAH.

Key words: *Marosatherina ladigesi*, embryonic development, larval stage, early ontogeny

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Corresponding Author: Jayadi, Department of Aquaculture, Faculty of Fishery and Marine Science, Indonesia Muslim University, P.O Box 90231 Makassar, South Sulawesi, Indonesia Tel: +6281355-355-901

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Marosatherina ladigesi is an endemic ornamental freshwater fish in South Sulawesi, Indonesia[1-6]. This fish is locally called the "beseng-beseng". *M. ladigesi* is already registered as threatened species in the International Union for the Conservation of Nature as a fish that will become extinct[7,8], so that these fish still exist in the waters of Indonesia. *M. ladigesi* has been reported on the bio-ecology, morphology and karyotype[2], population and ecology[3] reproduction[4], infestations ectoparasites[9], feeding habits[10], fecundity and egg diameter[11], genetic diversity[12], reproduction under different sex ratios[5], gonadal maturation with live feed[13], spawning and rearing of larvae[14]. However, research on the development of embryonic and larvae of *M. ladigesi* has not yet been studied.

Ontogeny stages of fish is essential to understand[15] because the embryonic and larval developmental processes vary among different types of fish[16,17]. Development in early stages of the life cycle changes intensely during the hatching process and larval morphogenesis phase[18-20]. Knowledge of ontology development on fish will help increasing our knowledge of embryonic and larval development in fish will assist in the optimization of larval rearing techniques. The study aimed to determine the development stages of embryonic and larval *M. ladigesi*.

MATERIAL AND METHODS

Maintenance of broodstock and larvae was conducted in the freshwater ornamental fish hatchery at Manggala village in the municipality of Makassar, while the observation of embryonic and larval development was done in the biology laboratory facilities of the Agricultural Polytechnic State of Pangkep. The study was conducted on 23 March to 17 October, 2016.

Collection of brood stocks and eggs: *M. ladigesi* brood stocks were obtained from the Bantimurung and PatunungAsue Rivers of the Maros regency, the Jennae and Gowa Lorong Rivers of the Pangkajene Kepulauan regency and the Asanae River of the Bone regency in South Sulawesi, Indonesia[14,15].The numbers of broodstocks were 125 females (4.8-5.6 cm length) and 100 males (5.1-6.4 cm length). They were maintained in a fibreglass tank (L×W×H:200×150×75 cm) with a water volume of 350 litres. The broodstock was soaked in 1 mg L^{-1} potassium permanganate ($KMnO_4$) for 10 min before stocking and fed twice daily with mixture of *Daphnia* sp., *Chironomus* sp. and *Culex* sp. Water was substituted up to 50% each week. Brookstocks were reared for 5 months. Mature gonads of brook stocks were selected and kept in a fibreglass tank (L×W×H: 130×80×60 cm) with 100 L water.50 females and 25 males of broodstocks were used in this study. The spawning method was followed[13]. The brood stock ratio was 2 females:1 male[5]. Artificial substrate from plastic rope was attached in the tank. A Sony underwater camera was used for monitoring the spawning time. The eggs were collected6 h after laying on the substrate.

Larval rearing: *Marosatherina ladigesi* larval rearing was carried out in a fibreglass tank (L×W×H:60×40×35 cm) with a water volume 20 litresfollowing[13]. The water quality parameters were as follows: Temperature (27-29°C), DO (5.8-8.4 mg L^{-1}), pH (6.2-7.5) and NH_3 (<0.007 mg L^{-1}). Up to 20% of the water was changed weekly. The larvae were fed twice daily with *infusoria*, *Daphnia s*p. and *Artemiasalina* nauplii.

Observations: Observations of embryonic development were performed every 6 h until the eggs hatched. Egg samples were observed with 5 eggs at a time. Larvae were observed at the ages of 0.3, 5, 7, 10, 15, 20, 25 and 30 DAH. Both embryonic and larval developments were observed under a microscope (Olympus CX23).

The experimental protocol is based of government regulation of republic of Indonesia No. 60 year 2007 concerning conservation of fish resource.

RESULTS

Embryonic development: *Marosatherina ladigesi* began mating at night and continued until morning, spawning usually occurred at that time. Fertilized eggs were translucent in colour, while unfertilized eggs were white in colour. Description of the embryonic development of *M. ladigesi* in Fig. 1.

The blastodisc stage represents the early process of embryonic development of *M. ladigesi* and begins at the moment of fertilization when the egg and sperm cells forms the zygote (Fig. 1-6). The fertilized egg forms a nucleus at the centre at 6 HAS (Fig. 1a). The nucleus is located at the edge (Fig. 1b). The cleavage stage consists of 4-8 cell divisions occurring before 12 HAS, 16 cells form ingat 12 HAS (Fig. 1c) and 32-64 cells forming at 24-30 HAS (Fig. 1d and e), cell multiplication occurred after 36 HAS (Fig. 1f). The morula stage is when the formed blastomeres solidify to become a blastodisc polar anima, which forms two layers of cells. The morula stage occurred after 42 HAS (Fig. 1g and h). The

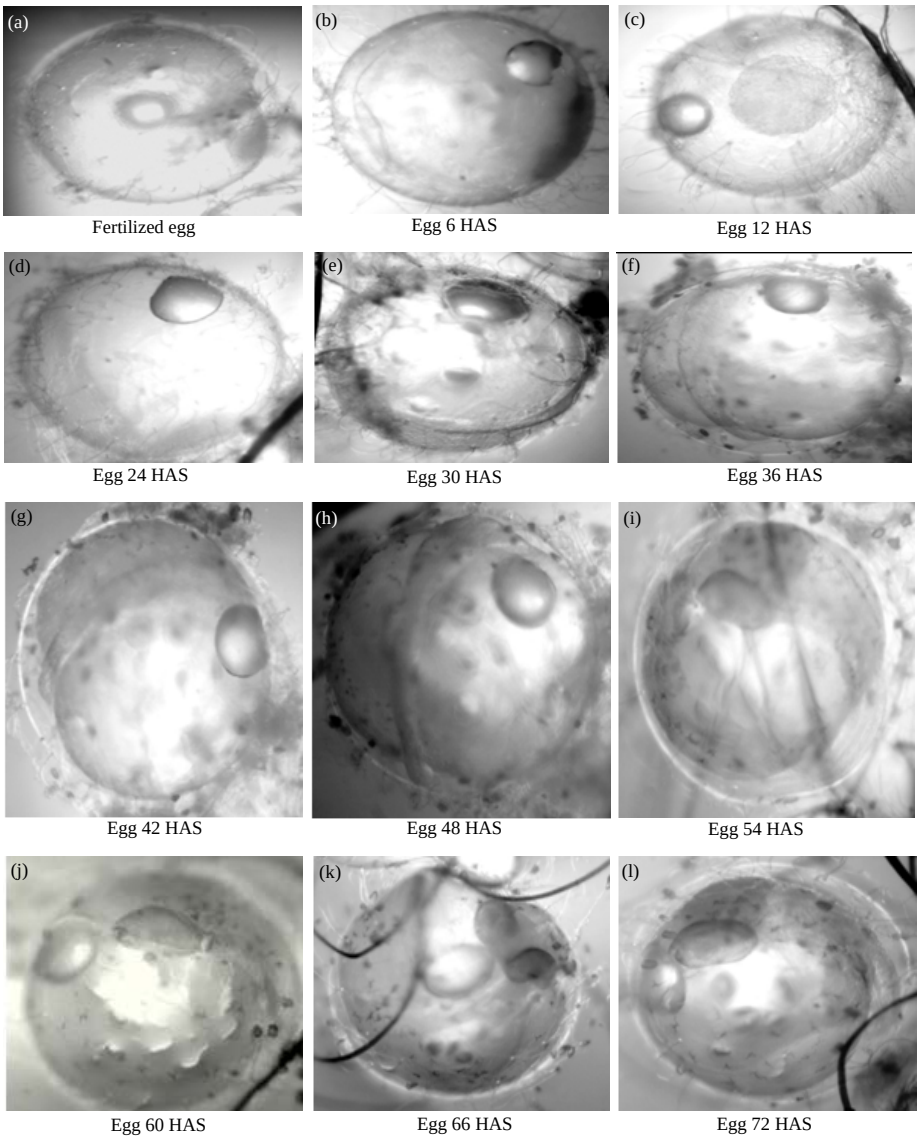

Fig. 1(a-l): Stage of embryonic development of *M. ladigesi*. No. 1-6: Blastodisc stage, No. 7-8: Morula stage, No. 9-10: Blastula stage, No. 11-12: Gastrula stage

blastula stage formed at 54-60 HAS (Fig. 1i and j). A very sensitive phase in the development of the egg occurs prior to the embryo stage, especially before reaching the blastula stage. Eggs that can pass through this critical phase can then continue to develop properly to achieve the embryo stage and hatch with a normal body shape. The gastrula stage formed after 72-78 HAS (Fig. 1k and l), in which the blastoderm cells begin to spread over the yolk, the end of this phase results in 100% coverage of the yolk cell by the blastoderm. The neurula stage occurred after 84-90 HAS (Fig. 1:13,14 and 15).

The segmentation stage occurred at 96-198 HAS (Fig. 2:16-33) in which the eye spots and body pigments in the head are visibly formed, the pectoral fin is forming, the rudimentary head and tail are visible and the notochord becomes detectable. Hatching occurred at 204 HAS or 8.5 DAH (Fig. 3a).

Larval development: The development of *M. ladigesi* larvae is described in Fig. 3.

The newly hatched larvae have a closed mouth, transparent body and a melanophore on the top of the head and the upper and central body (Fig. 3a-c). The yolk sac and eyes were formed after the segmentation phase. The yolk volume at hatching was 0.568 µL. The mouth was open after 65-78 h (3 DAH)(Fig. 3c-f) and the yolk reserves were used for 5 DAH (Fig. 3d-f).

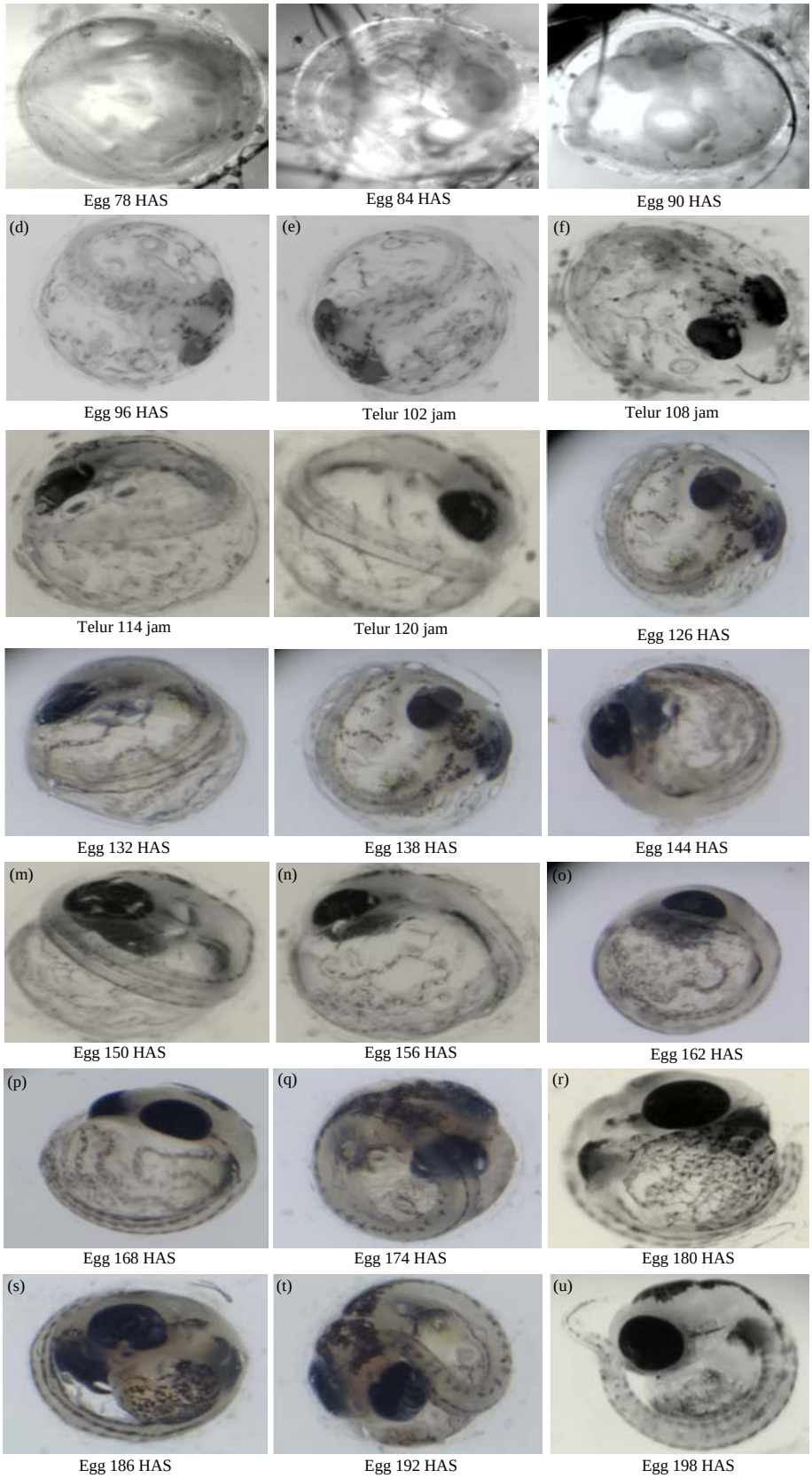

Fig. 2(a-u): No.16-33: Segmentation stage of embryonic development of *M. ladigesi*. The segmentation stage already formed eye spots

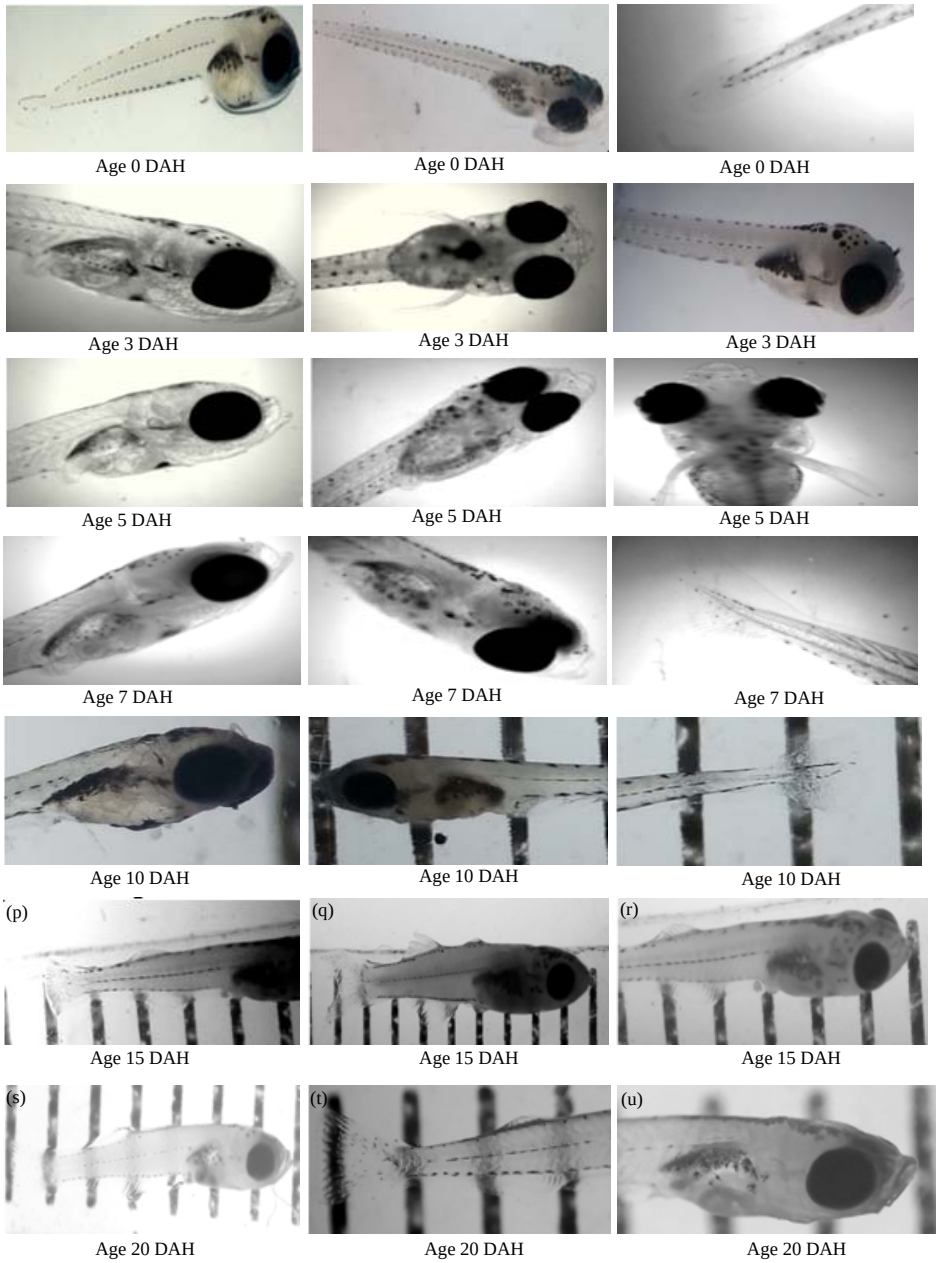

Fig. 3(a-u): Stage larvae development of *M. ladiges*. Free flexion phase at the age of 7 DAH, flexion phase at the age of 10 DAH and post flexion at the age of 5 DAH

The newly hatched of larvae had pectoral fins and a merged structure including the dorsal fins, anal fin and caudal fin, which was round in shape (Fig. 3a-c) and changed in the free flexion stage (7 DAH) (Fig. 3k-p). The separation process of the three fins occurred at 10 DAH, which is called the flexion stage (Fig. 3p-r), the total separation occurred at 15 DAH and is called the postflexion stage (Fig. 3g-i). The body muscle segments were observable at 20 DAH (Fig. 2:10-12). After 25 DAH (Fig. 3.13-15), the melanophore was still present and the colour of the fish was translucent white. The number of melanophores decreased with increasing fish age. The changes in the larval body in the postflexion stage can be seen in the fish body, which became typical in form (Fig. 3: 16-18). The newly hatched larva had a total length of 4.45-5.21 mm and 4.23-5.03 mm standard length.

DISCUSSION

Embryonic development: The results of embryonic development studies on *M. ladigesi* have been described in

Fig. 1. *Marosatherina ladigesi* has eggs that adhere to the substrate until they hatch. The results of this study have provided an overview of the embryonic development of the morula phase formed after 42 HAS, the blastula stage formed at 54-60 HAS, the gastrula stage formed after 72-78 HAS, the neurula stage occurred after 84-90 HAS, the segmentation stage occurred at 96-198 HAS and hatching occurred at 204 HAS or 8.5 DAH.

Marosatherina ladigesi is typical of the teleosts in its general development under natural condition and represent behavioural reproductive strategy that serves to protect the eggs from water drift until hatching and which can be related to environmental conditions[17]. Adhesive eggs have also been reported in *Corydoras aeneus*[17], *Acipenserbaerii*[21] and *Carassius auratus*[22].

When the embryonic stage reach the segmentation stage (Fig. 2:16-33) they became very active and the chorion was softened by enzymes that were secreted from the hatching glands[23]. The secretory activity of the hatching gland cells is controlled by various environmental factors such as temperature and oxygen concentration[24]. The growth rate of embryonic differs among species. The eggs of *M. ladigesi* hatched after 204 h or 8.5 DAH (Fig. 1), whereas the egg incubation period for *Melanotaeniasp lendida* is 151-152 h and for *Melanotaenianigrans* is 155-159 h at 25-27°C[25], the incubation period for *Oreochromisniloticus*is 2.3 days at 34.5°C[26]. The embryonic stage of *Melanotaenia* sp. for 127 h and 4 min[27], while in *Sahyadriadenisonii* it lasts 36 HAS[19], the embryonic stage of *Daniorerio* was found to be 96 HAS[28]. The eggs of *Neopomacentruscyanomos* hatched at 3 HAS[29] and *Mystacoleucuspadangensis* eggs were hatched 19 HAS[30]. The differentiation of larval depends on the species, egg size and temperature[23].

Larval development: The description of larval development on *T. ladigesi* has been described in Fig. 3. Larval development in fishes can be divided into the yolk sac, preflexion, flexion and postflexion stages[23,30,31]. Stages of development of larva *M. ladigesi* namely the yolk sac stage lasts for 5 DAH, preflexion begins at 7 DAH, flexion begins at 10 DAH and postflexion occurs at15 DAH (Fig. 3). The newly hatched *M. ladigesi* had closed mouths which opened after 65-78 h (3 DAH) and the yolk volume at hatching was 0.568 (µL). The eyes of the larvae began to form and grow before hatching (Fig. 2:16). The mouth of *M. ladigesi* larvae opened and they began eating at 3 DAH while the yolk reserves were still available. The fish body shape changes in the preflexion stage (Fig. 3:12). The preflexion stage when the total body length is 4.81-5.31 mm (7 DAH), then the fish enters the flexion stage (Fig. 3:12-15) in which the total length is 5.42-6.27 mm (10 DAH) followed by the postflexion stage (Fig. 3:16-18) in which the total length is 6.57-7.32 mm (15 DAH).

The newly hatched larvae have reserves and the mouth is still closed. After 3-DAH larvae start looking for food from outside while yellow reserves are still available. This species uses a combination of endogenous and exogenous resources in its early life. This is similar to *Limanda yokohama*[32], *Oxyeleotris marmorata*[33], *A. baerii*[34], *Chelon labrosus*[35] and *Trichogaster fasciata*[36]. The transition from endogenous to exogenous feeding in larval stage is a critical process[37,28]. At this stage, significant changes in the larval body occur before exogenous feeding[19]. This period involves functional and morphological changes in the internal organs and tissue systems and particularly in the digestive system[38,39], as a consequence, there is high mortality in the larval phase of *M. ladigesi* ontogeny at 5 DAH. This high rate of mortality also occurs in *M. nemurus*[40] and *C. labrosus*[35].

The yolk sac reserves were depleted at 5 DAH was found for *M. padangensis*[30]. However, after hatching, the duration of the food reserves differs among species, for example, in *Mugil cephalus* it lasts for 14 DAH[41], in *Mystusnemurus* for 3 days[42], in *Acanthopagrus cuvieri* for 8 DAH[43], in *A. baerii* for 9 DAH[18] and in *Neolissochilus hexagonolepis*, the reserves last for 16 DAH[44]. The differences in yolk absorption rate depend on the yolk size and environmental factors such as temperature and dissolved oxygen level[45]. Higher temperatures results in more rapid absorption of food reserves, for example in *Spratus auratus*[46] and the *Lota-lota*[47]. The eyes of the larvae began to form and grow before hatching for example *Pseudobagrus ichikawa*[48], *M. nemurus*[40] and *Brycon cephalus*[49]. However, in some species the eye pigment develops after hatching, which in *Chanos* occurs after 36 h and in *Lates calcarifer* and *Siganus* sp. after 24 h[50]. The eye pigment in newly hatched *Sahyadri adenisonii* is also not yet formed[51]. In *Betta splendens*[52] and *M. padangensis* larvae, it occurs at one HAS[30].

In the preflexion stages, there are changes in the body, morphology, metabolism and behaviour that depend on the fish species, larval size and rearing conditions[16]. The development on the ventral side of the notochord in the flexion stage is associated with the tail fin. In this stage, the process of separation of the pectoral, caudal and anal fins occurs to facilitate swimming activities.

This was the first study to describe the key development stages of embryonic and larvae of *M. ladigesi* will help us to optimize the problem of egg hatching rate and as a basis in feeding the larvae to promote growth and survival as well as for led us to a sustainable management of endemic celebes

rainbow fish in hatchery. Future studies are obviously affecting factors of embryonic and larval development for restocking of this species and can meet the demand of ornamental fish market in Indonesia.

CONCLUSION

Marosatherina ladigesi eggs are very adhesive and do not float and the eggs hatch to larvae at 204 HAS or 8.5 DAH at 29±1°C. This study revealed that the flexion stage occurred after 10 DAH and the postflexion stage after 15 DAH. The mouth was open after 65-78 h (3 DAH) and the yolk sac reserves were used for 5 DAH. Adequate knowledge of the embryonic development in this species will help us to optimize egg hatching rates and a better understanding of the stages of larval development can serve as a basis for rearing and feeding larvae for improving growth and survival.

SIGNIFICANCE STATEMENT

The study discovered the the key development stages of embryonic and larvae produced by domesticated broodstocks of *M. ldigesi* and provide the overview of embryonic development starting from morula phase (42 HAS), the blastula stage (54-60 HAS), gastrula stage (72-78 HAS), neurula stage (84-90 HAS), the segmentation stage (96-198 HAS) and end at the hatching (204 HAS or 8.5 DAH). Also accentuate the larvae stages of *M. ladigesi* namely the yolk sac stage lasts for 5 DAH, preflexion begins at 7 DAH, flexion begins at 10 DAH and postflexion occurs at15 DAH. This study will help researchers to uncover critical areas of the phase of ontogeny in *M. ladigesi* that many researches were not able to explore whereas, results of this study are also helpful in the development of cultivation and conservation of *M. ladigesi*. Thus the best theory with following feature of embryonic and larval development may be arrived at provide benefits in the development of cultivation and conservation of *M. ladigesi*.

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