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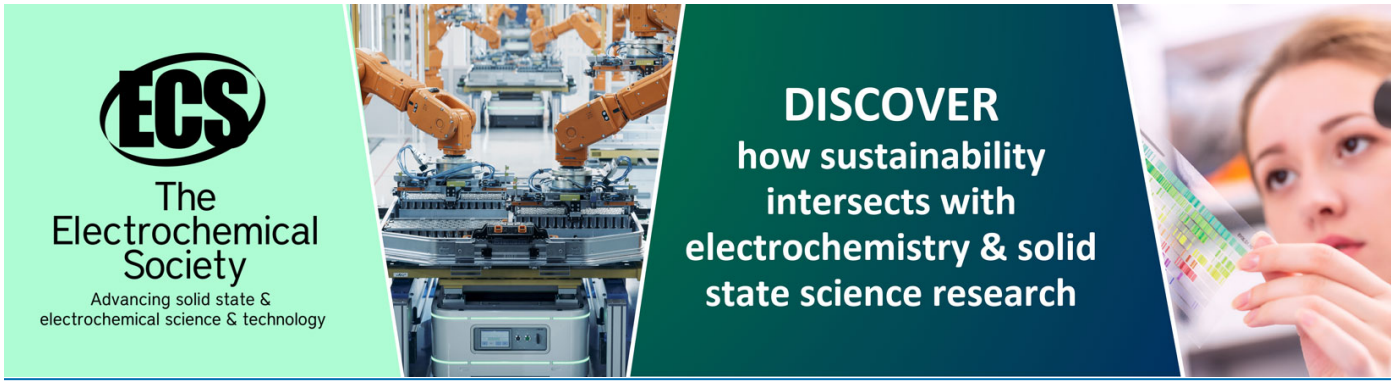

The development of adapted marmorated medaka *Oryzias marmoratus* (Aurich, 1935) at ex-situ habitat

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Abstract. Marmorated Medaka (*Oryzias marmoratus*) is a small endemic fish species of Malili Lakes, South Sulawesi, Indonesia. The fish is a valuable commodity as an ornamental fish. However, their population in wild is threatened with extinction and has been categorized as vulnerable by IUCN. An effort to conserve *O. marmoratus* by ex-situ breeding is necessary for its sustainability. Research Center for Limnology-LIPI has succeeded to take the fish from Lake Towuti and adapted them in an ex-situ habitat (laboratory) (2017–2018). Research on the development to adapt *O. marmoratus* outside their natural habitat has been conducted in 2019-2020. The sex ratio of male: female was 1:2 (1:2.223±0.638). During mass spawning, the fish was able to reproduce seven times in 37 days which the dark moon phase. Egg diameter (mm) was 1.193±0.017. Reproduction viability was very good. Fertilization and hatching rates were 100% and 98.149±3.347%, respectively. Length of Incubation Period (LIP) (days) was 7.286±1.329, and the larva size (mm) was 3.3±0.483. Survival Rate on seven days (SR_7) was 97.02112±5.510 % with larva size of 5.5±0.707. Juvenile size (mm) at 16 weeks was 33.6±5.661 (26–42). *Oryzias marmoratus* can be developed in ex-situ habitats and still needs further research.

Keywords: adapted fish; development; ex-situ habitat; *Oryzias marmoratus*

1. Introduction

Oryzias marmoratus or Marmorated medaka belonging to the family Adrianichthyidae, Subfamilia Oryziinae, which has 34 species distributed in the world [1]. The fish is endemic in Malili Lakes, South Sulawesi, Indonesia, which is distributed in Lake Towuti, Lake Mahalona, Lake Matano, Lake Lanto, and Wawontoa [2, 3]. In Malili Lakes were also distributed *Oryzias profundicola*, *O. celebensis*, *O. matanensis*, *O. woworae* in Muna Island, and *O. hadiatyae* in Masapi, while *O. nigrimas* and *O.orthognatus* were found in Lake Poso, Central Sulawesi [2−5].

Oryzias marmoratus is benthopelagic, schooling is not a migratory fish, has a small size (usually about 4-5 cm), and has an attractive color. Adult fish have a distinctive color body, which is olive green or brownish-yellow. This blue-eyed Medaka has a side part of the body that contains dark dots arranged in an orderly manner like marble with several spots, so this fish was recognized as Marmorated Medaka (figure 1). The head and abdomen tend to be dark, and there is a dark spot at the base of the tail. The base of the caudal fin, the tip of the pectoral fin, and the front side of the anal fin are bright yellow to orange, while the outer side of the anal and caudal fins are dark. Due to their attractive body shape and color, they have an economical value as an ornamental fish and as a potential fish model for several

experiments [6−8] and as a consumed commodity by the people around the Lake Towuti, therefore the exploitation of the Medaka are intensive [9−11]. Their population in the wild is threatened to extinction and by IUCN has been categorized as vulnerable species [12] or Near Threatened [13]. An effort to conserve *O. marmoratus* by ex-situ breeding is necessary for its sustainability. The results of the ex-situ development can also be used for various purposes. According to Puspitasari and Suratno [14], the fulfillment of the number of biotas can only be fulfilled if the biota has been breeding outside of their natural habitat. However, ex-situ development information of the fish is rarely reported. Some research on *O. marmoratus* fish have been reported such as adaptability to seawater, its potential as an ornamental fish, the survival of the transportation from the natural habitat, and early adaptability at controlled conditions [6, 8, 15−17].

Ex-situ development is a technology that could be done to conserve certain species, especially endemic ones, from extinction. This effort is necessary to reduce exploitation activity. Ex-situ development of endemic fish has been successfully carried out by the Research Center for Limnology-LIPI and other institutions. Several types of fish that have been successfully developed such as Celebes Rainbow (*Marosatherina ladigesi*), endemic Rainbowfish of Papua (*Glossolepis incisus*, *Melanotaenia boesemani*, *Melanotaenia praecox*), *Rasbora argyrotaenia*, *Tor douronensis*, *Melanotaenia parva*, *Amphiprion ocellaris,* and others [18−23]. This research aims to evaluate the further development of *O. marmoratus* that have been adapted at controlled conditions or ex-situ habitat at the aquatic laboratory of Research Center for Limnology-LIPI. The component of evaluating such as the growth and reproduction viability of the adapted Marmorated medaka.

Figure 1. *Oryzias marmoratus* [17].

2. Materials and methods

The exploration activity and adaptation of *Oryzias marmoratus* from Lake Towuti, South Sulawesi has been carried out in 2016-2017 at the Aquatic Laboratory of Research Center for Limnology-LIPI [16, 17]. This research was carried out at Aquatic Laboratory of Research Center for Limnology-LIPI in 2019-2020 using the adapted *O. marmoratus*, which is the progeny of the parent were collected from Lake Towuti since 2017.

The observation component includes the size of the fish at a certain age, the size of broodstock, sex ratio, egg diameter, and the reproduction viability of the fish in the ex-situ habitat. Observation of the length size of larvae was conducted randomly on fish larvae aged 0 days, 1, 2, 4, 8, 12, and 16 weeks using a little glass container with a scale (mm) at the bottom, while to measure the length of 33 broodstock samples using a ruler in mm scale, and observation of egg diameter was using a Digital Caliper Model No. 111-011 on 33 laid eggs sample at the substrate. The sex ratio was observed in 13 populations whose population members were between 10 to 43 fish.

Observation of reproduction viability was carried out on a fish population of 45 broodstock with a sex ratio (male: female) of 1: 2. Broodstock was reared in a fiber tank dimension 2.1x1.1x0,5 m, the water level of 35 cm, equipped with an aeration system with temperature (26.32−26.58)^{o}C, pH (8.61−8.63), and Dissolved oxygen/DO (8.08−8.2) mg/L, respectively. The broodstock was

acclimatized for one week before running. During the observation, the fish were fed twice a day by frozen bloodworm and pellet, while the larvae were fed by a fine powder of pellet.

The artificial substrate of gray or black color plastic material (*raffia*) was used in the rearing system to keep the newly laid eggs **(**figure 2). Observations were conducted every day for 37 days. The small aquarium (25x25x20) cm was used for hatching and rearing the larvae with temperature, pH, and DO were (26.25−27.28)^{o}C, (7.44−7.66) and (8.18−8.28) mg/L, respectively. Spawning tanks and hatcheries aquariums were placed in a roofed room. The reproduction viability observed included the number of spawning (NOS) and interval of spawning (IS), the number of ovulated eggs (NOE), fertilization rate (FR/%), hatching rate (HR/%), length of the incubation period to hatch (LIP/days), and survival rate for 7 days (SR_7) (table 1) [18]. Measurements of temperature, pH, and Dissolved Oxygen used the WQC (Water Quality Checker) measuring instrument [Horiba, Japan].

Table 1. The components of reproduction viability observation of adapted *O. marmoratus*.

No	Observation Components	Description
1	Number of Spawning (NOS)	Total spawning during 37 days
2	Interval of Spawning (IS)	The number of days among spawning times
3	Number of Ovulated Eggs (NOE)	Amount of eggs for each spawning
4	Fertilization Rate (FR, %)	The percentage of the amount of fertilized eggs to the total ovulated eggs.
5	Hatching Rate (HR, %)	The percentage of the number of larvae to the fertilized eggs
6	Length of the incubation period (LIP, days)	The number of days required for hatching
7	The survival rate for 7 days (SR_7, %)	Percentage of the number of larvae at 7 days to total hatched larvae

Figure 2. Rearing fiber tank with the substrate (arrow) and hatching aquarium.

3. Results and discussion

The length of larvae aged 0 days to 16 weeks is shown in table 2. Shortly after hatching the larvae size was 3.3±0.483 mm and at 16 weeks old was 33.6±5.661 mm. So that the daily growth for 16 weeks was 0.271 mm/day. Said and Mayasari [24] reported that the daily growth of adapted Celebes Rainbow

Marosatherina ladigesi in 12 weeks was 0.21−0.29 mm/day. According to Said and Mayasari [17] that the daily growth of *O. marmoratus* during the adaptation period in ex-situ habitat was 0.286 mm/day in 5 weeks, while in this research the daily growth of 4 weeks was 0.283 mm/day. This shows that the daily growth of larvae of *O. marmoratus* in both conditions was similar. However the size of larva in this condition (table 2) is smaller than the size of larvae in the adaptation period, that larvae aged 3 days, 5 weeks, and 11 weeks olds were 5.7±0.83 mm; 15.7±1.25; and 26.0±1.50, respectively that fed by nauplii Artemia since the larva was 7 days old. This is presumably due to the difference in the type of feed given. Therefore for the further development of *O. marmoratus* an appropriate type of feed is needed.

Table 2. The length size (mm) of larvae of adapted *O. marmoratus* at 0–16 weeks olds.

Length size (mm)	Age (weeks)							
	0	1	2	4	8	12	14	16
Average	3.3	5.5	9.0	11.24	15.71	24.5	28.3	33.6
SD	0.483	0.707	0.816	1.715	1.38	2.173	4.692	5.661
Range	3−4	5−7	8−10	9−14	14−18	21−27	24−38	26−42

The observation of 13 populations shown the sex ratio (♂:♀) of *O. marmoratus* was 1: 2.1±0.357 (1:1.5−1:1.233) (tabel 3) or 1:2. In general, schooling fish has a character that the number of males is lower than the female, the male body size is bigger and more colorful than the female. The phenomena like the Celebes Rainbow or Papua Rainbowfish [18, 19, 25, 26]. This is thought to be related to the natural behavior of schooling fish and the composition of the sex ratio in reproduction. So the observation of viability in this research (table 4) used the sex ratio was 1:2. In this study, the size of the broodstock was 43.172 ± 4.997 (34-52) mm. The size is similar to the size range for adult *O. marmoratus* and other *Oryzias* (*O. javanicus*, *O. matanensis*, *O. nigrimas*) which is about 5 cm [2, 14, 27, 28].

Eggs diameter of adapted *O. marmoratus* was 1.193±0.357 (1.18−1.22) mm. The egg size is large, it is similar to the egg diameter of the Celebes rainbow (*Marosatherina ladigesi*) was about 1.10–1.15 mm [29, 30] and *O. javanicus* was about 1.1 mm [14]. Several studies have reported that an interesting biologic aspect of Medaka is that spawning occurs in oviviparus, large egg size and spawning occurs throughout the day [6]. The eggs diameter of *Oryzias* is larger than the Papua Rainbowfish (*Glossolepis incissus*, *Melanotaenia ayamaruensis*, *M. ajamaruensis*) which are about 0.9 mm (*have not published*). From this phenomenon is suggested that the egg size of Oryziinidae has larger than Melanotaeniidae.

Table 3. Sex ratio, broodstock size (mm), eggs diameter (mm) of adapted *O. marmoratus.*

Component	Sex Ratio (♂:♀)	Broodstock size (mm)	Eggs diameter (mm)
Average	01:02.1	43.172	1.193
Standard deviation	0.357	4.997	0.017
Range	1:1.5−1:2.333	34−52	1.18−1.22

Observations of reproduction viability carried out for 37 days with a sex ratio (♂:♀) of 1: 2. From the mass, spawning was obtained the number of spawning was 7 times with the spawning period was 5.286±3,904 (2–12) days (table 4) [8] also found that *O. marmoratus* does not spawn every day. The number of eggs (NOE) was 55.286±34.043 (25−105), and the number of the larva (NOL) was 54.714±34.253 (23−104). Fertilization rate (FR) and Hatching Rate (HR) were 100% and 98.149±3.347

(92−100)%, respectively. The Length of Incubation Period (LIP) was 7.286±1.329 (6−9) days and SR_7 was 97.021±5.51 (87.72−100) (table 4).

Table 4. The number of spawning (NOS), the interval of spawn (IS), number of ovulated eggs (NOE), number of the larva (NOL), FR (%), HR (%), SR_7 (%), and LIP/days of adapted *O.marmoratus*.

Components	NOS (time)	IS (days)	NOE	FR (%)	HR (%)	NOL	LIP (days)	SR7 (%)
Average	7	5.286	55.286	100	98.149	54.714	7.286	97.021
Standard deviation		3.904	34.043	0	3.347	34.253	1.329	5.51
Range		2−12	25−105		92−100	23−104	6−9	87.72−100

In several trials, it was seen that single/individually spawning showed a failure to spawning and even causing the death of the female parent. Due to the phenomenon so this research used bulk spawning. Researched on Celebes Rainbow, that mating individually with a ratio of 1:1 for male and female, the fish failed to spawn and ended with the death of the female. Spawning goes well in mass spawning with a ratio of male:female 1:2 [24]. Total spawning was 7 times, that six times of spawning which took place during the dark moon phase (waning crescent to waxing crescent) and only one time took place when the moon is waning gibbous (17th in Hijriyah calendar) with the minimum number of eggs, namely 25 (table 4). The large number of eggs obtained during spawning took place in the dark moon phase. It is suggested that the spawning of *O. marmotaus* is related to the lunar system. The spawning of *O. marmoratus* is better on the dark moon phase than the third quarter to the first quarter moon phase.

In this study, it was seen that all spawning was followed by hatching. The FR value was very good, all of FR was 100%, which means that all eggs spawned were fertilized. This shows that the sex ratio in the spawning of male: female 1:2 is very suitable according to the sex ratio in the population. The HR value in this study was so high, which shows that almost all eggs spawned have hatched with the LIP was 7.286 days (table 4). This is better than the viability of the adaptation period that HR only 61.14 (17.24−100)% with an average of LIP was 8 days and as 33.33% failed to hatch [17]. The LIP of *O. marmoratus* was shorter than the LIP of *O. sarasinorum* that 11 and 14−20 days [14, 31]. From the results, it is suggested that *O. marmoratus* has adapted well and could be developed in an ex-situ habitat.

4. Conclusions and suggestions

Marmorated Medaka (*Oryzias marmoratus*) had a large egg size. Reproduction took place well at a sex ratio of 1:2, which the dark moon (waning crescent to waxing crescent) phase. Reproduction viability was very good on FR, HR, LIP, and SR_7. Therefore *O. marmoratus* has adapted well and can be developed in an ex-situ habitat.

Further research is needed to optimize the fish products such as on the viability of reproduction at certain times, as associated with the season (rainy and dry) or the lunar system. Research is also needed to increase the number of eggs, accelerate growth or size with the treatment of optimum feed conditions and environment.

Acknowledgments

This research was carried out at Aquatic Laboratory, Research Center for Limnology-LIPI. Special thanks to Dr Sekar Larashati, Dr Hidayat, Syahroni and Ismail for their help.

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