Reproductive ability of endemic species of Lake Ajamaru 
*Melanotaenia ajamaruensis* Allen & Cross, 1980 on spawning at various sex ratio

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**Abstract.** *Melanotaenia ajamaruensis* or Ajamaru Rainbowfish is endemic species of Lake Ajamaru, Papua, Indonesia. It belongs to Rainbowfish group, with data deficient status. They have attractive color, especially on male and has potential as an ornamental fish, but the percentage of male is low. A study was conducted to find out their reproductive ability at various sex ratios (male:female = 1:1; 1:2; or 1:3) with two replications for 40 days (January-February 2021) at Research Center for Limnology, LIPI. Observations were also conducted on sexual maturity age, egg diameter, size at a certain age. The ratio of 1:3 have the highest value on number of spawning (NoS): 25 times, number of eggs (NoE)/female: 54 eggs, number of larvae (NoL)/female: 49 larvae, fertilization rate/FR (%): 97.16; hatching rate/HR (%): 97.93; larvae survival at seven days/SR7 (%): 90.34, and eggs incubation time/EIT (days): 5.24. The sexual maturity on 8 months old. Egg diameter (mm): 0.98±0.043, larvae size (mm) at (0 day), and at 1, 2, 4, 8, 12 weeks old were: 3.2±0.68; and 6±0.74; 11.70±1.77; 19.48±1.86; 21.65±2.64; 25.0±2.94 respectively. Daily growth rate 0.26 mm/day That is indicates that *M ajamaruensis* could adapted well to ex-situ habitat and the reproduction could takes place with a small number of males (1:3).

1. **Introduction**

Indonesia has a high biodiversity of freshwater fish distributed from Sumatra to Papua and many are endemic [1-3]. A recent report from [4] states that Indonesia has 565 endemic freshwater fish species. *Melanotaenia ajamaruensis*, which is only found in Lake Ajamaru Papua, is one of the freshwater endemic fish species listed on the list. This fish belongs to the Rainbowfishes group in the Melanotaeniidae family. *Melanotaenia ajamaruensis* has a size of about 11 cm, schooling fish. Like the other Melanotaenia, this fish has a good appearance having a color that is generally reddish brown on the back and front half of the body with yellow or tan gradations on the posterior with a series of red-brown horizontal stripes on the sides. Live fish sometimes appear with a metallic blue to yellowish base color or green with yellow longitudinal stripes (fig.1). Generally, the color is more obvious in male individuals than in female fish, but the number of male individuals is lower. This appearance is very similar to *M. boesemani* which is also endemic to the same lake. The main difference between the two species is in the calculation of the soft spines for the second dorsal and anal fins [2, 5] and the appearance
of a more pronounced yellow coloration in *M. ajamaruensis*, whereas *M. boesemani* is relatively stronger in the blue appearance. The advantages it has cause this fish to have potential as an ornamental fish and has an economical values.

*Melanotaenia ajamaruensis* described by Gerald Allen and Norbert Cross in 1980, that the fish is one of the species that Marinus Boeseman's collection when he an expedition to several areas in Papua in 1954 - 1955 [5]. The biological data of this fish is still very limited and according to the IUCN that this species has a status with data deficien so that it requires research and conservation of it [6] while in nature it faces threats, including biological threats from the Toraja snake head fish and Cyprinus which are a introduced fish [7]. This condition is feared to cause extinction. Therefore, research and efforts to conserve the *M.ajamaruensis* is need to be carried out, among others, by means of conservation at the species level in ex-situ habitats.

*Melanotaenia ajamruensis* have been able to reproduce in ex-situ habitat. For further step in ex-situ habitat is needed to improve the quality or reproduction of the fish by several methods including manipulation environment of rearing, both the physical-chemical environment of the media or biological manipulation such as for the effectiveness of using brood stock. In addition, to anticipate the condition of a low percentage of male fish and to make reproduction more efficient at ex-situ habitats, research is needed for the efficiency of the reproductive process. For this reason, in this reproductive research, biological manipulation of the sex ratio was carried out to obtain the optimal composition so that the fish could reproduce optimally and use male broods efficiently. In general, the sex ratio (between male and female individuals) is one of the most basic parameters in demography for the survival of a population of species in the future breeding [8].

Several studies on the effect of sex ratios on the reproductive ability of fish in cultivation have been carried out, including zebra fish, Anabas swamp fish, Helostoma, Arapayma [9-12]. [13] have also conducted research on the effect of sex ratios on the reproduction of single species of *Marosatherina (Marosatherina ladigesi)* that endemic of the Maros river, South Sulawesi, Indonesia, and also on the native Indonesian fish, Lake Maninjau, *Rasbora argyrotaenia* [14]. However, the reproductive pattern of *M. ajamaruensis* in ex-situ habitats with various sex ratios between males and females has not been reported.

Therefore the aims of this study to obtain the optimal of brood stock sex ratio at ex-situ habitats to reproduction and obtaining optimal fish progeny of *M. ajamaruensis*. The parameters of reproductive viability which included number of spawning, number of eggs, number of larvae, survival of the 7 days old of larvae and the time required for hatching refer to [15, 16]. In addition, to obtain data on *M. ajamurensis*, research was also conducted on the size of the egg diameter, the size of juveniles at a certain age period, and early reproductive age or the age at which sexual maturity is reached. The results of this study are expected could encourage some future studies about the fish.

![Figure 1. Melanotaenia ajamaruensis](image-url)

2. Materials and Methods
In this paper, there are several separate studies, namely reproductive viability at various sex ratios, observation of egg diameter size, size of larvae at a certain age, and early reproductive age. All research
was carried out at the Aquatic Laboratory, Limnology Research Center, Cibinong. The study was conducted for 40 days (January-February 2021). The brood stock used were around 18 months old which was a gift from the Laboratory of Research Institute for Ornamental Fish Culture, Ministry of Maritime Affairs and Fisheries Republic of Indonesia, Depok on July 2020. The fish were hatched at the laboratory with the brood stock being adapted fish from the exploration of Lake Ajamaru several time ago.

This reproductive research was carried out individually using three different sex ratio treatments, there is male to female ratio (♂:♀) namely A (1:1), B (1:2) and C (1:3) with two replications, so there are 6 aquariums were used. Brood stock were reared in an aquarium with a size of 45x45x40 cm filled by water as high as 20 cm which was equipped with an aeration system. The water in the aquarium was aerated for 3 days before being the fish were reared. The entire aquarium is placed in a roofed room without walled. Each brood stock was measured in length and weight (table 2). During the observation period, the brood fish were fed ad libittum by frozen Chironomus twice a day (morning and evening).

Before retrieval of spawning data, the fish were acclimatized in the spawning aquarium for 7 days. Egg collection and observation parameters

Observation of reproductive viability refers to [15, 16] (table 1). Artificial substrate of gray or black color plastic material (raffia) was used in the rearing system (aquarium) to keep the newly laid eggs for 40 days. The small aquariums (30x25x20) cm were used for hatching and rearing the larvae until 7 days old. The hatcheries aquariums were placed in a same room to the aquarium of brood stock. The reproduction viability observed was carried out daily included the number of spawning (NoS), the number of ovulated eggs (NoE), fertilization rate (FR/%), hatching rate (HR/%), eggs incubation period to hatch (EIP/days), number of larvae (NoL), and survival rate of larvae for 7 days old after hatching (SR7) (%). Data analysis was done descriptively.

Calculation of the (NoS), (NoE) and (NoL) produced were carried out for all spawning that took place in each treatment (from 2 replicates). Then averaged based on the number of female brood stock in each treatment. All data on two replicates for each treatment were collected, so the data displayed is for each female (table 3). While the value FR, HR, SR7 and EIP data are the average values of all spawning that took place in each treatment (table 4). Characteristics of fertilized eggs, among others, are transparent and have a strong adhesion to the substrate.

Rearing of larvae

The number of hatched larvae was counted and kept in the hatchery aquariums. During the rearing period, the larvae are fed with cooked chicken egg yolks (since the larvae are 2 days old). After the larvae were 7 days old, then additional types of feed were added with nauplii of Artemia. Hereinafter the larva be transferred to the other rearing system.

Table 1. The description of parameter of reproductive ability observation of M. ajamaruensis

| No. | Parameter of reproductive viability | Description |
|-----|-----------------------------------|-------------|
| 1   | Number of Spawning (times)        | NoS         |
| 2   | Number of Ovulated Eggs (eggs)    | NoE         |
| 3   | Number of Larva (larvae)          | NoL         |
| 4   | Fertilization Rate (%)            | FR          |
| 5   | Hatching Rate (%)                 | HR          |
| 6   | Survival Rate at 7 days (%)       | SR          |
| 7   | Egg Incubation Time (days)        | EIT         |

Observation of egg diameter, larval size, early reproductive age, rearing of progeny

Observation of the egg diameter was carried out on 50 total laid eggs sample at the substrat were collected from several spawning which took place in August 2020. Measurements were carried out using a Digital Caliper Model No. 111-011. While the observation of the length size of larvae was
conducted randomly on fish larvae with a number of between 25-70 samples aged 0 days, 1, 2, 4, 8, and 12 weeks using a little glass container (8x2x2 cm) with a scale (mm) at the bottom. Larvae rearing is done by means of fish larvae that are kept in the aquarium until the age of 7 days and are fed with cooked chicken egg yolks. After 7 days of age the fish larvae were transferred to rearing system using a hapa (net) measuring 0.5x0.5x0.4 m³ which was placed in a fiber glass tank measuring 2.1 x 1.1x0.6 m³. In this system, the larvae were given additional Artemia feed until they were 1 month old (30 days), then continued with fine pellet commercial feed.

Early reproductive age or age of sexual maturity observations of *M ajamaruensis* fish were carried out on laboratory hatched juvenile fish that took place in August 2020 which were reared in round fiberglass tubs with a diameter of 1.2 m and a height of 0.7 m. Observation of reproductive sustainability begins when the fish are 6 months old from hatching by placing an artificial substrate into the fish population.

3. Result and Discussion

The average size of the brood stock *M. ajamaruensis* used in this study is listed in table 2. Overall, it can be seen that the size of the male fish is larger than the female. Table 2 also shows that the size of the female in treatment A was the largest, followed by treatment C and then by treatment B (A>C>B). This condition tends to occur of the Melanotaeniidae family Rainbowfish such as *Melanotaenia bosemani, M. praecox, M. lacustris, M maccullochi* and *Golosolepis incissus* [15] [17]. This phenomenon presumed that a characteristic of benthopelagic fish and schooling fish as well as Sulawesi rainbow fish *Marosatherina ladigesi* and Marmorated Medaka *Oryzias marmoratus* [13][16][18]

| Sex Ratio (♂:♀) | A (1:1) | B (1:2) | C (1:3) |
|-----------------|---------|---------|---------|
| Male            | 2       | 2       | 2       |
| Female          | 2       | 4       | 6       |

Table 2. The average size of brood stock at each treatment

| Size of Brood stock | A (1:1) | B (1:2) | C (1:3) |
|---------------------|---------|---------|---------|
| Length (cm)         | Male    | Female  | Male    | Female  | Male    | Female  |
|                     | 7.4±0.141 | 7.35±0.212 | 7.25±0.354 | 7.01±0.131 | 7.4±0.141 | 7.08±0.392 |
| Weight (g)          | Male    | Female  | Male    | Female  | Male    | Female  |
|                     | 4.79±0.028 | 4.51±0.332 | 4.20±0.481 | 4.18±0.596 | 4.59±0.06 | 4.34±0.552 |

Table 3. The number of male and female broodstocks, NoS (times), NoE (egg), NoL (larvae) of each female

| Sex Ratio (♂:♀) | NoS (times) | NoE (egg) | NoL (larvae) |
|-----------------|-------------|-----------|--------------|
| A (1:1)         | 10          | 5         | 63           |
| B (1:2)         | 14          | 3.5       | 118          |
| C (1:3)         | 25          | 4.2       | 324          |

In table 3, it can be seen that the total number of spawning (NoS) of each female was highest in treatment C, which was 25 times during 40 days. This shows that the continuity of spawning takes place better in treatment C that spawning used as much as 62.5% of the time (days) period. It seen that spawning takes place every 1.6 days. While the spawning period in treatment B took place every 2.5
days and treatment A every 4 days. In treatment C, it was also seen that the highest NoE and NoL of each female were 54 eggs and 49 larvae respectively. This shows that the male: female 1: 3 treatment was very effective to produce the number of spawning, total number of eggs and number of larvae and progeny. Two advantages of the results shown by this study that are very supportive to anticipate the low percentage of male individuals of *M. ajamaruensis* population and requires only a small amount of time to spawning and to produce of a large number of survive progeny.

The NoE of B treatment was relatively small compared to other treatments. This is thought to be related to the body size of the broodstock. Small body size tend to have less fecundity or number of eggs as seen in Celebes Rainbow *Marosatherina ladigesi* [13] [19]. According to some literature of Melanotaeniidae [20] that fecundity is related to body size of the broodstock. Generally, the larger body size of the productive brood in one species, the higher the fecundity, as of some species of Papua Rainbowfish (*Glossopelis incisus, M. arfakensis, M. fluvialitis, M.parecox*). According to [21] that the direct influence on the total number of eggs are the size of the female parent's body and the size of the egg diameter.

**Table 4.** The value of FR(%), HR(%), SR7(%) and EIT (days) of each treatment

| Value of | Sex Ratio (♂:♀) | A (1:1) | B (1:2) | C (1:3) |
|---------|-----------------|---------|---------|---------|
| FR (%)  | HR (%) | SR7(%) | EIT (days) | FR (%)  | HR (%) | SR7(%) | EIT (days) | FR (%)  | HR (%) | SR7(%) | EIT (days) |
| Average |         |         |           |         |         |         |           |         |         |         |           |
|         | 97.0   | 96.67   | 83.85     | 5.6     | 97.29   | 96.74   | 87.72     | 5.64    | 97.16   | 97.93   | 90.34     | 5.24     |
| Sd      | 6.749  | 10.541  | 20.315    | 0.84    | 5.047   | 7.669   | 14.566    | 0.842   | 5.377   | 20.68   | 18.27     | 1.052    |
| Range   | 80–100 | 66.67–100 | 50–100 | 5–7 | 83.33–100 | 72.72–100 | 66.67–100 | 4–7 | 83.33–100 | 54.54–100 | 71–100 | 4–7 |

The FR values in the three treatments were similar, namely both at 97% (table 4). The FR value indicates the ability of sperm to fertilize the ovulated eggs of the female. These data indicate that the amount of sperm from one male has the same ability to fertilize eggs ovulated by one, two, or three females. It is presumed that the ratio between sperm is considered balanced to the number of eggs that are spawned, so that fertilization and then hatching rate is better. Further research such as the relationship of the amount of sperm, body size, age, and ability to fertilize a greater number of females of *M. ajamaruensis* is needed.

**Table 5.** Size of eggs diameter(Ø) and length size of larvae (mm) at a certain age old (weeks)

| Size (mm) | Eggs (Ø) | Age of larva old (weeks) |
|----------|----------|-------------------------|
|          |         | 0 | 1 | 2 | 4 | 8 | 12 |
| Average  | 0.98     | 3.20 | 6.00 | 11.70 | 19.48 | 21.65 | 25.00 |
| Sd       | 0.043    | 0.68 | 0.74 | 1.77 | 1.86 | 2.64 | 2.94 |
| Range    | 0.88–1.06 | 2–4 | 5–7 | 9–15 | 17–23 | 17–27 | 19–30 |

The HR values of all treatments are almost similar. The C treatment had the highest of SR7 value, which was 90.34% (table 4). The EIP values in the three treatments were almost the same, namely in the range of 5 days. This value is smaller than the EIP of several species of Melanotaeniidae in a captive that about 7 (6.33-8.67] days[15], also of *M. ladigesi* was 9.8 ± 0.42 hari and of *O. marmoratus* that was
7.286±1.329 (6–9) days [13, 16]. The HR value and EIT can be influenced by various factors such as the intrinsic factor of the embryo (fish species) and external factors or the environment in which the embryo is located, such as water, temperature, light intensity, water movement, dissolved gases (among others), dissolved Oxygen of the rearing water, and the treatment when the embryo is transferred from the spawning aquarium to the hatchery aquarium. The difference in species allows for differences in the quality of both eggs and the ability of embryo to grow. Another factors that can affect the incubation period is the condition of the egg such as chorion thickness, chorion resistance, and the effectiveness of chorion softening enzymes. In addition, each species has a varying and very specific embryonic growth rate [22–25]. The temperature range of rearing water was 25-26 °C, the pH of the water was 6.5-7 is suggested that are the appropriate range to support the HR and EIP of M. ajamaruensis.

The average size of the ovulated egg diameter of M. ajamaruensis was 0.98±0.043 (0.88–1.06) mm (table 5). Several studies of Melanotaeniidae (M. arfakensis, M. duboulayi, M. splendida) showed that egg diameters were 0.81–1.12 mm [20]. While the Oryzias egg diameter was relatively larger such as O marmoratus was 1.193 (1.18–1.22 mm) and O. javanicus was about 1.1 mm [21, 16, 26]. From the phenomenon, there is a tendency that Melanotaeniidae have smaller egg diameters than Oryzidae.

The length size of the larvae age 0 (days) is the size of the larvae on the day of hatching, that is 3.2 ± 0.68 (2-4) mm (table 5). The size of this larva is almost the same as that of O. marmotaus larvae at the same age (0 days) that is 3.3 mm [16]. The size achieved by M. ajamaruensis at 2 weeks of age was 11.70 which is almost the same as the size achieved by M. parva at 3 weeks of 11.93 mm [27] and [28] reported that the size of M. parva on 7 days was about 7.79 mm that by microscopic analyzed. Table 5 shows the length size of larva at 12 weeks old is 25±2.94 (19-30) mm. Based on these data, it can be estimated that the daily growth of M. ajamaruensis larvae was 0.26 mm/day. The daily growth of several Melanotaeniidae species (G incisus, M. bosemmani, M. paraecox, M. lacustris, M maccullochi) in ex-situ habitats is around 0.25-0.26 mm/day [15]. The daily growth of M. ajamaruensis is almost the same to the other fish at ex-situ habitats, [14] reported that the daily growth of adapted Celebes Rainbow Marosatherina ladigesi in 12 weeks was 0.21–0.29 mm/day. According to [29] that the daily growth of O. marmoratus during the adaptation period in ex-situ habitat was 0.286 mm/day in 5 weeks. The data of the growth also show that M. ajamaruensis has been well adapted to ex-situ habitat.

In this observation, it was found that the early age of M. ajamaruensis to be able to reproduce or sexual maturity was 8 months old. This condition was indicated by the ability of the 8-month-old fish population to produce transparant eggs and have strong adhesion to the substrate, and the eggs were able to hatch at EIT in about 5-7 days. The freshwater fish Aplocheilus lineatus has the sexual maturity on 6 months old and the optimal reproduction age is 6-10 month [30]. In line with these conditions, the sexual maturity of freshwater shrimp Macrobrachium sintangense is 6 months old but the optimal reproductive period was 7-12 months [31]. Therefore It is necessary to find out the optimal age of M. ajamaruensis brood stock to produce optimal progenies.

Sex ratio study on zebra fish conducted by [9] that found that total egg production of the fish increased in line with the increase in the number of females used in spawning. In addition, several previous studies on Melanotaenia bosemmani showed that the best sex ratio of male:female to obtain the most larvae was 2:5 [un published]. The best sex ratio (male:female) of M. ladigesi was 1:2, Rasbora argyrotaenia was 2:1; and O. marmoratus was 1:2 by mass spawning pattern and in Helostoma temminckii fish was 4:1 [11], Arapaima gigas was 1:1 [12], while the sex ratio treatment had no effect on the reproduction pattern of Anabas fish [10]. From this phenomenon it is suggested that the sex ratio for reproduction is relatively specific of each fish species. It is presumed that each species has its own reproductive strategy which is related to the condition of the sex ratio in its natural population to maintaining its offspring. According to [24] that the main objective of the reproductive strategy is to maximize the reproducitively active offspring in relation to the available energy conditions and the hope to maintain the existence of the species. Research on the sex ratio of fish for optimal reproduction is considered necessary in order to find the best composition between males and females. The best composition will be very useful in determining the effectiveness of brood fish such as in terms of the need for optimal sperm count to fertilize a number of eggs. In addition, fish breeding behavior is
relatively varied. If the ratio of the number of male fish is relatively large or of different sizes it can interfere with the implementation of spawning eggs because the aggressive condition of the male fish affects the comfort of the female fish when reproducing occur, as was the case in the Irian rainbow fish hybridization experiment [15].

Sex ratio of broodstock can influence the reproductive behaviour of a species [12]. At a ratio (male:female = 1:3) *Melanotaenia ajamaruensis* has a relatively large number of females. It is presumed that the increase number of female brood stock, there will be a process of mutual influence between them to reproduce. According to [32] [33] that in fish reproduction generally have steroid and prostaglandin reproductive hormones that will be used as endogenous signals (in the body) between the reproductive tract and the brain. These hormones are also useful as exogenous signals (hormonal pheromones) that will synchronize or mediate reproductive or maturation activities, gametes in teleostey and/or spawning interactions between two individuals or among other of the same species. Based on this argument, mass spawning is thought to be better than individual spawning. In this study it was seen that the number of spawning, number of eggs, number of larvae was highest in the large number of female parents [male:female =1:3]

4. Conclusion

The endemic species of Lake Ajamaru Papua, Indonesia *Melanotaenia ajamaruensis* has the ability to reproduce at a male:female sex ratio of 1:3 which means one male is able to fertilize 3 female individuals. The composition has the highest number of spawning, fertilization rate, number of eggs and number of larvae. *Melanotaenia ajamaruensis* has eggs with a relatively small diameter. The fish have been able to grow in ex-situ habitat with a daily growth of 0.26 mm/day and the sexual maturity age is 8 months. Further research on *M ajamaruensis* is required.

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