

The reproduction viability of Indonesian endemic fish *Oryzias marmoratus* (Aurich, 1935) fed by *Spirulina*

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Abstract. *Oryzias marmoratus* is one of the ornamental aquarium fish species that live endemic to Towuti Lake, South of Sulawesi, Indonesia. However, the fish has been categorized as near threatened by IUCN. A current report showed the fish could be cultivated. This research aims to improve the reproduction quality in cultivation by using artificial feed containing *Spirulina* (A=0, B=3, and C=6) % dry weight. The study was conducted with two replications using an aquarium (80 x 40 x 40 cm) for 40 days (October-December 2020) at Research Center for Limnology-LIPI. Each aquarium contains 21 broodstock (female: male = 2:1). Observation parameters are the total number of spawning/ToS (time), ovulated eggs number/OEN, fertilization ability/FA (%), hatching ability/HA (%), number of larvae/NoL, the egg of incubation time/EIT (day), survival of larvae seven days old after hatching/SR7 (%). Based on descriptive analysis, the use of feed C (containing 6% *Spirulina*) produced the highest values of ToS, OEN, %HA, NoL, and SR7 (%) were ten times; 89 eggs; 89 larvae, 100%; and 98.89±3.51% respectively. *Spirulina* could increase the reproduction viability and is presumed to enhance feed efficiency of *Oryzias marmoratus*, but the optimum levels have not been obtained.

1 Introduction

Oryzias marmoratus with the famous name Marmorated medaka is one species of the Medaka group that belongs to the Subfamilia Oryziinae, family Adrianichthyidae. These benthopelagic and schooling fish is endemic on Towuti Lake and other lakes in the Lakes of Malili group, South of Sulawesi, Indonesia [1, 2]. Adult fish has a beautiful color on the fins, and on both sides of their small body sometimes look transparent and sometimes olive-green color with a regularly black spot distributed like marble (Fig.1). Therefore *O. marmoratus* could be used for aquarium fish or an experimental material for various research like the other Medakas [1, 3-6]. In addition, the fish has a function as a protein source for the local communities of Lake Towuti, and the fishing activity of the fish is very

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intensive [7, 8]. According to IUCN information, the fish has the conservation status as vulnerable or Near Threatened species [9, 10]. The condition can be anticipated by conservation efforts necessary to carry out both in-situ or ex-situ development (domestication) or cultivation. A current report that *O. marmoratus* can survive, adapt and develop in ex-situ habitats or cultivation [6, 11]. A further step is needed to improve the quality of the fish. Quality improvement can be carried out in several methods, including maintenance environment of rearing, both the physical-chemical environment of the media cultivation and treatment of the feed given, or other manipulations. Feed treatment can be done by adding certain substances to the feed given. *Spirulina* is one of the additives materials that can be applied in the manipulation of fish feed.

Spirulina is a single/colonized type of blue-green algae (micro-algae) that contains high protein. Besides, it also contains chlorophyll, carotenoids, phycobilin pigments (phycocyanin and phycoerythrin), essential acids, various vitamins, minerals [12–15]. The nutrient contents of *Spirulina* are excellent as a source of food, health, and cosmetics of humans, as well as feed. In an aquaculture system, *Spirulina* is applied to fish feed ingredients to improve the quality of fish, such as reproduction as well as growth appearance, survival, taste, or the color performance of ornamental fish. Microalgae have an essential role in the aquaculture system. The use of microalgae can be indirectly or directly related to the impact of nutrients on various aquaculture organisms [16].

Research on the uses of *Spirulina* in feed for ornamental fish, consumption fish or shrimp has been widely carried out such as chichlid, koi, guppies, Tricogaster, goldfish, carp, tilapia, catfish, or giant prawns [17 – 24], and others. In general, the overall use of *Spirulina* has a good effect on these commodities such as color appearance, taste, growth, reproduction, and feed conversion. A trial of feed containing *Spirulina* on increasing reproduction of the endemic fish *O. marmoratus* has not been reported. It is expected that the addition of *Spirulina* on the feed will increase the viability of reproduction of *O. marmoratus*. This research aims to study the performance of reproductive viability of *O. marmoratus* fish fed by *Spirulina* and obtain information on the optimum dose of *Spirulina* in feed for the highest reproduction viability of the fish.



Fig. 1. *Oryzias marmoratus* (Photo: DSSaid)

2 Materials and methods

2.1 Preparation of broodstock and rearing aquarium

The study was conducted for 40 days (October-December 2020) with two replications. The number of broodstock and replications used in this research were adjusted to the number of broodstock available. The broodstock used was about 11 months old, a collection of Research Center for Limnology-LIPI Laboratory. Spawning was carried out by mass spawning, of each 21 broodstock a ratio of female: male equal to 2:1 (14 female and 7male). The sex ratio of the fish in this research refers to [6]. The broodstock was measured and then reared in an aquarium measuring 80x40x40 cm, filled with a height of 25 cm, and

equipped with an aeration system. The fish were acclimatized for one week to this rearing system.

2.2 Feed preparation and feeding

The primary feed used was commercial feed (pellets). At the same time, the *Spirulina* added to this feed was dried powder *Spirulina* cultured from the Planktonology Laboratory, RC for Limnology, Indonesian Institute of Sciences (LIPI). Commercial feed is ground and then regenerated using a feed-making machine. Three categories of feed were made, namely Feed A (primary feed, 0% *Spirulina*/control), Feed B was (basic feed+3% *Spirulina* per kg of feed), and Feed C (basic feed + 6% *Spirulina* per kg of feed). After re-pelleting, the feed is dried in an open room without direct sunlight. Then the feed formed is reduced in size using a blender and then filtered to get feed according to the size of the fish mouth. The amount of feed for each aquarium was weighed (g). The observation fish were fed *ad libitum* in the morning and evening. After 40 days of observation, the remaining feed was weighed. The amount of feed consumed (Table 2) is the result of subtraction between the initial feed and the last feed.

2.3 Observation of reproductive viability

Observation of reproductive viability refers to [6, 24]. The dark color of plastic material (*raffia*) was used as an artificial substrate to keep the newly laid ovulated eggs. The observation was carried out daily for 40 days. The eggs' substrate was moved to a bit of aquarium (25x25x20) cm for hatching and larval rearing until seven days old. These aquariums are also housed in the broodstock room. The viability of reproduction observed consists of the total of spawning (ToS), the ovulated eggs number (OEN), fertilization ability (FA/%), hatching ability (HA/%), eggs incubation time to hatch (EIT/days), some larvae (NoL), Rasio of hatching to spawning (%) and survival of the larvae for seven days old after hatching (SR7) (%) (Table 1). Data analysis is done descriptively.

Table 1. Parameters of reproduction viability [6, 25].

No.	Parameters of viability	Explanation
1	Total of Spawning/ToS (time)	Total Number of spawning for 40 days
2	Ovulated Eggs Number/ OEN (egg)	Total Amount of ovulated eggs of all spawning
3	Fertilization Ability/FA (%)	The ratio of fertilized eggs to total ovulated eggs
4	Number of Hatching/NH (time)	The total of hatchings that took place from the number of the spawning
5	Ratio of Hatching to Total Spawning/NH of ToS (%)	The percentage of the amount of hatching to the total spawning has occurred
6	Hatching Ability/HA (%)	The percentage of hatched larvae to total fertilized eggs
7	Number of Larvae/NoL (larvae)	Some larvae that have hatched
8	Egg of Incubation Time/EIT (day)	Total time (days) required from spawning to hatching
9	Survival 7 days old after hatching/SR7 (%)	The ratio of larval survival at seven days to the initial number of larvae

3 Result

The average size of male and female broodstock and the amount of feed consumed in each treatment are shown in Table 2. The reproduction viability values of *O. marmoratus* are shown in Table 3.

Table 2. Broodstock size of *O. marmoratus* and the amount of feed consumed.

Sex	The broodstocks size and amount of feed of each treatment								
	A			B			C		
	Length (mm)	Weight (g)	ΣFeed (g)	Length (mm)	Weight (g)	ΣFeed (g)	Length (mm)	Weight (g)	ΣFeed (g)
♂	44.43	0.71		42.43	0.85		40.4	0.73	
Sd	3.41	0.18		2.76	0.16		4.28	0.11	
Range	38–48	0.56–0.99		40–48	0.60–0.99		38–48	0.60–0.89	
			18.84			18.18			16.88
♀	40.0	0.66		41.0	0.70		40.24	0.65	
Sd	2.37	0.12		3.77	0.09		3.21	0.03	
Range	38–46	0.55–0.88		36–48	0.60–0.88		37–46	0.60–0.73	

Table 3 shows that *O. marmoratus* fed with *Spirulina* content of 6% (C treatment) has the highest ranking on 6 of the nine observed viability parameters (Table 3). Those were produced the highest of ToS, OEE, NH, NoL, HA, and SR7 compared to other feed treatments. The FA and % Rasio hatching of spawning (NH of ToS) value has the same value, which is 100% each, while the EIT value (day) is longer than the others.

Table 3. The value of reproduction viability of *O.marmoratus* for 40 days.

No	Parameters of viability	Feed treatment			The rank of the value of treatment		
		A	B	C	A	B	C
1	ToS (time)	5.5±0.71	8.5±0.71	10±0.0	3	2	1
2	OEN (eggs)	44.5±7.78	66.5 ±2.12	89±2.83	3	2	1
3	FA (%)	100	100	100	R	R	R
4	NH (time)	5.5±0.71	8.5±0.71	10±0.0	3	2	1
5	NH of ToS (%)	100	100	100	R	R	R
6	HA (%)	86.87±13.65	92.71±13.68	100±0	3	2	1
7	NoL (larvae)	39±6.75	61.6±1.97	89±2.83	3	2	1
8	EIT (days)	7.60 ± 1.82	7.63 ± 1.30	7.80 ± 1.87	1	2	3
9	SR7 (%)	96 ± 8.94	98.44 ± 4.42	98.89 ± 3.51	3	2	1

R = have the same values

4 Discussion

The length average of broodstock used in the three treatments was almost the same size, about 40 mm (Table 2). These sizes are similar to the size of an adult *O. marmoratus* has used by [6]. The size of the male is more significant than the female. According to [1, 3, 4, 6] that *O. marmoratus* is about 4-5 cm in length, and the female is smaller than males.

The fish of C treatment consumed the least amount of feed compared to others that which was 16.88 g, followed by treatments B (18.18 g) and A (18.84 g) (Table 2). On the other hand, the number of ToS and OEN in treatment C was mainly followed by treatment B then A (Table 3). Those number shows that the amount of feed consumed is not directly

proportional to the number of spawning eggs produced. These data indicate that the use of *Spirulina* in feed can increase the feed efficiency of *O. marmoratus*. Inline to [18, 26, 27] results that *Poecilia reticulata*, *Carassius auratus*, and *Clarias gariepinus* fed a diet with a higher *Spirulina* content had a good effect on the value of feed conversion and growth.

Broodstock size in C treatment was relatively smaller than in B treatment and almost the same as the A treatment (control). However, the ToS, OEN of C treatment was more than B and A treatment. The ToS and OEN in treatment C were also higher than the results of [6], which showed that *O. marmoratus* broodstock size was $43,172 \pm 4,997$ (34-52) mm produced only seven times of ToS in 37 days and 55 (25-125) ovulated eggs. Generally, a larger body size had higher fecundity [28]. The results of previous studies also showed the positive correlation of body weight with fecundity like in *Puntius ticto* [29], *Neolissochilus sumatranus*, and *Tor douronensis* [30]. This study also showed that feed containing higher *Spirulina* had affected increased fecundity (shown by OEN) and spawning number (ToS) of *O. marmoratus*. According to [14], all yellowtail chichlid fed with *Spirulina* diets was significantly higher on the production of total eggs than the fish fed diet control. So that indicated that the inclusion of dietary *Spirulina* meal has the potential to enhance reproduction, the performance of growth, and the coloration of the fish. Similarly, [31] reported that feeding containing 8% of *Spirulina* has better performance on growth, fertility, gonadosomatic Index, gonadal quality, and coloration on ornamental fish *Xiphophorus helleri*. This phenomenon shows that feed containing *Spirulina* has the same effect on *O. marmoratus*.

In this study, it was seen that the FA (%) and the ratio of NH to ToS (%) values for all treatments had the same value (100%) (Table 3). The result of [6] that as many as 45 broodstock *O. marmoratus* (male: female = 1:2) produced a spawning frequency of 7 times 37 days. All samples have FA and Rasio of NH to ToS, which are 100% each. The FA and ratio of NH to ToS values of each 100% indicates that all eggs produced by females are fertilized by the sperm of males, which results in good quality embryos which all hatch to produce larvae. This ratio confirms that the sex ratio for the optimum reproduction of *O. marmoratus* is male: female = 1: 2.

The HR and SR 7 in C treatment were the highest that are 100 and $98.89 \pm 3.51\%$, respectively. According to [14] that yellowtail chichlid (*Pseudotropheus acei*) fed with *Spirulina* diets was significantly higher on hatching rate (%), and that was increased the survival and growth rate of *Clarias gariepinus* [27] and enhanced the production of progeny and growth performance of Kenya cichlid (*Maylandia lombardoi*) [32]. This value shows that *Spirulina* inclusion in the feed improved its growth and survival rate, growth performance, progeny production, and skin coloration.

The EIP value (days) in treatment C (7.80 ± 1.87) was slightly longer than the EIP value of B (7.63 ± 1.3) and A treatments (7.60 ± 1.82) (Table 3). Increased content of *Spirulina* in the feed, there is a tendency to increase the EIP time. Feed containing *Spirulina* presumed affects the hatching process of the eggs (embryos). It is presumed that the mineral content (such as Calcium) of *Spirulina* has strengthened the condition of the embryo chorion, namely the permeable protective layer of the embryo. The research results [33] in poultry that the addition of Calcium to the feed has strengthened the quality of poultry eggs, and [34] was reported that the addition of Calcium in the rearing water affects the hatching process of hybrid channel catfish eggs. Calcium provides specific needs in the embryogenesis process of fish [34].

In general, the results of this study indicate that the feed with 6% of *Spirulina* has enhanced the reproduction viability value than the 3% *Spirulina* treatment and control on the number of spawning, number of eggs, hatching ability, number of larvae produced, and survival rate of larvae seven days old after hatching. This study also showed that *Spirulina* in the feed had increased the feed efficiency of *O. marmoratus*.

Spirulina is micro-algae that contains high protein (up to 70% dry weight) amino acids, lipids, fatty acids, photosynthetic pigments, carotenoids, various vitamins, minerals, and other ingredients [15, 26] that are essential for, *i.e.*, reproduction development and growth performance. The results of this research indicated that the fish are in good health condition. These conditions indicate that the fish get complete nutrition from the feed given. The protein content in the feed provides the best feed quality so that protein is used for reproduction, somatic growth, and feed efficiency. According to [35], the protein, fatty acids, and lipids of *Spirulina* are the main constituents of egg yolk, and essential fatty acids content provide energy for fish spawning. The rate of vitellogenesis, development, and maturation of oocytes are affected by protein and essential fatty acid, and B vitamin from *Spirulina* will improve the performance of fish reproduction [35].

5 Conclusion

Spirulina in feed up to 6% has increased reproduction viability in the components of a total number of spawning, number of ovulated eggs, hatching ability, number of larvae produced, and survival of larvae seven days old after hatching. The optimal level of *Spirulina* in feed has not been obtained to increase the highest reproduction viability of *O. marmoratus*. There was also a tendency that the *Spirulina* had increased the feed efficiency of *O. marmoratus*.

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