

Technical Notes: Breeding of a single hatching fry to an adult in medaka

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Using conventional methods of breeding, the survival rate of hatching fry of medaka is about 50%. However, the survival of a single fry is important in experiments of developmental biology such as transgenesis and chimera formation. In addition, the fast growth rate of founder fry which have been produced in these experiments is also important to obtain adult fish in a shorter period of time. The breeding method described here has been developed as a simple and convenient method leading to a higher survival rate and faster growth by the author based on the advice of Dr. Y. Taguchi (National Institute of Radiological Sciences). Using this method, more than 80% of fry develop to adults around 2 months after hatching.

I. General conditions

1. *Container*: Use plastic containers such as mouse cages (215W × 320D × 140H mm).
2. *Temperature*: Maintain temperature at 26°C. Temperatures ranging from 25 to 28°C are suitable.
3. *Light*: Natural light from windows or illumination by fluorescent lighting is appropriate. Lighting conditions of 14 hours' light and 10 hours' darkness are generally used for daily spawning.
4. *Water*: Tap water should be kept in tanks longer than one day.
5. *Feeding*: Live baits are generally better for growth than nonlive baits. Here, however, a method using nonlive baits is described, because live baits are troublesome to prepare.

Medaka prefers live baits such as *Paramecium* and blind shrimp larvae. *Paramecium* is a suitable feed for fry soon after hatching. Blind shrimp larvae are suitable feed for fry older than ten days to two weeks' old and also for adult fish. Feeding of live baits in addition to nonlive baits results in faster growth and lower mortality of fry, earlier sexual maturation, and an increased number of eggs in one spawning in comparison with feeding of nonlive baits only.

In any kind of feed, it is important to give a suitable quantity of feed which is consumed quickly by the fish. Overfeeding should be avoided, and leftover feed should be removed. Leftover feed floating on the surface of the water can be easily removed by absorption to a piece of paper towel which is placed on the water surface. A membranous layer formed on the water surface due to bacterial proliferation can also be removed using the same method. Leftover feed at the bottom of aquaria can be removed using a small net or by pipetting.

Frequency of feeding is twice a day (morning and evening).

II. Preparation and maintenance of aquaria

1. Water is filled into clean containers at a depth of 4 cm (ca. 2.5 l). Add 400–500 ml of green water. These aquaria are suitable for breeding of adult fish and young fish older than one month old.

To prepare the green water, keep green water collected from different sources of the fields in containers illuminated in the laboratory. The green color of water becomes weak at first, and then recovers in some containers. Such green water is maintainable in the lighting conditions of the laboratory and can be used for breeding of fish. Green water may spontaneously occur when fish are bred in clear water under the lighting conditions in the laboratory.

2. For breeding of fish less than one month old, aquaria should be "conditioned". To prepare the "conditioned" aquaria, breed an adult male fish in a newly prepared aquarium described in (II-1) for 3–4 days. From this, a stable water condition is attained where the green color becomes darker and microorganisms proliferate. Do not use a female fish for "conditioning", as this may cause contamination of experimental fry with eggs spawned by the female.
3. Water in aquaria should be partly changed. Do not change the entire aquarium at one time. Sludge of the bottom of aquaria should be removed using a small net or by pipetting. The

green color of the water will fade during the course of breeding. If fading occurs, exchange aquaria with fresh ones.

III. *Breeding of fry*

A. Hatching to 2 days

1. Fry start swimming actively soon after hatching. Introduce these fry into the "conditioned" aquaria (II-2) as soon as possible after they have reached this stage.
2. Introduce a small amount of boiled chicken egg yolk dispersed in water using a pipette. (The boiled chicken egg yolk is mashed, subdivided into microtubes, and stored at -20°C until use.) The dispersion is carried out by grinding the yolk in water in a glass mortar. If feeding is delayed, the mortality of fry increases.
3. Add water based on the volume lost by evaporation. Do not change water.
4. The number of fish in an aquarium should be not more than 20.

B. Up to 2 weeks

1. Boiled chicken egg yolk dispersed in water and baby fish food "E" for egglayers (Tetra, Germany) are given together or separately in one day.
2. Add water based on the volume lost by evaporation. Do not change water.
3. The number of fish in an aquarium should be not more than 20.

C. Up to 4 weeks

1. Feed baby fish food "E" for egglayers.
2. Change $1/3$ of the volume of water every 5 to 7

days. Add water based on the volume lost by evaporation.

3. The number of fish in an aquarium should be decreased to not more than 10. Variations in the body size may appear among fry in this stage. If such variations are found, divide the fry into two groups according to their body size and breed each group in different aquaria. In the later stages of growth, the same procedure should be employed.

D. Up to 6 weeks

1. Feed powdered TetraMin which is prepared by grinding flakes of TetraMin (Tetra, Germany) using a mixer and has been filtered through a $150\text{ }\mu\text{m}$ mesh.
2. Change $1/2$ of the volume of water every 3 to 4 days.
3. The number of fish should be not more than 7.

E. Up to 2 months

1. Feed powdered TetraMin which has been filtered through a $300\text{ }\mu\text{m}$ mesh.
2. Change $1/2$ of the volume of water every 2 days.
3. The number of fish should be not more than 7.

F. Beyond 2 months

Fish grow to adults around 2 months after hatching.

1. Feed powdered TetraMin which has not been filtered.
2. Change $1/2$ of the volume of water every 2 days.
3. The number of fish should be not more than 5.