

Introduction

Fish larvae show a high survival rate when they can get enough nutrients especially at the time from hatching up to 1 week post-hatching. We observed a smaller frequency of food intake in see-through medaka larvae under normal breeding conditions, possibly due to an impaired vision. Therefore, see-through medaka larvae are required to have a special environment in which enough feed is close to each larva. However, overfeeding using commercial feeds leads to deterioration of breeding water. In this case, breeding and taking care of medaka larvae is very time consuming. Here, we show a breeding protocol using *Paramecia caudatum* as a suitable feed for larvae. This is available for breeding larvae of see-through medaka, and also many kinds of other medaka.

Table 1. Comparison between *Paramecia caudatum* and commercial feeds.

	Advantages	Disadvantages
<i>P. caudatum</i>	No deterioration of breeding water	Necessity for paramecia management
Commercial feeds	No feed management (easily available by purchase)	Necessity for bihourly cleaning tanks, because of avoiding water deterioration due to overfeeding

Materials and Methods

1. Preparation of *P. caudatum*

1.1. Devices and tools

Name	Use application	Remarks
<i>Paramecium caudatum</i> *	Live feeds	The culture solution containing <i>P. caudatum</i> is directly added into the tank in order to have an abundant amount of paramecia. This solution doesn't have any lethal effect on larvae.
EBIOS* (Asahi food & healthcare co., ltd.)	Nutrients for bacteria	Bacteria are feeds for <i>P. caudatum</i> . Paramecia stock solution already contains bacteria, there is no need for preparation.
Deionized water	For culture and stock solution	Tap water in NIBB is also suitable. Tap water in other institutes needs to be checked.
500 mL-conical flask	For culture	Two bottles are enough to breed 100 larvae.
Silicon plug	For covering culture flask	<i>P. caudatum</i> grows under aerobic conditions.

* NBRP medaka? in Japan can send those to your lab.

1.2. Culture of *P. caudatum*

Culture solutions should be prepared for both use and storage. Here is the preparation method for one bottle of culture solution of *P. caudatum*.

1. Add one tablet of EBIOS and 450 mL of deionized water into 500 mL flask
2. Cover the flask with silicon plug and further wrap the plug with aluminum foil to avoid evaporation during autoclave
3. Autoclave the flask
4. Cool the flask at room temperature
5. Add 50 mL of stock culture into the flask in clean bench

The culture solution containing grown *P. caudatum* can be used for feeding after 3-5 days.

2. Breeding of see-through medaka larvae

2.1. Devices and tools

Name	Use application	Remarks
Brine shrimp	Feeds for larvae with 2-3 wk age	
Deep round container	Breeding tank	63 x 32 mm [dia. x h] for 1-20 larvae, 90 x 45 mm or 95 x 58 mm for 21-80 larvae
Black sheet	To block light from bottom side	Both paper and plastic is suitable.
Plastic transfer pipette	To clean water	Larva can be sucked in, if a larger pipette is used. See-through medaka are more sensitive to handling and scooping.

2.2. Procedure for breeding see-through medaka

To sum up following procedure:

A breeding environment needs to be created in which larvae can frequently meet *P. caudatum* and the amount of breeding water needs to be adjusted for producing the condition of high-density paramecia (the water should not be too much).

Day 1

Put the deep round container on the black sheet to block light from the bottom side. STII larvae show a higher survival rate and growth under this condition, compared to the condition using a white sheet (unpublished data of Chisada).

1. Transfer hatching larvae to deep round container by using a transfer pipette

2. Add paramecia solution into the tank until 1.5 cm in depth, and leave it until you go back home
3. Exchange breeding water before you go back home
 - 3-1. Take up as much as possible of breeding water and debris on the surface and the bottom by using a plastic pipette. The larvae should still be able to swim.
 - 3-2. If there is still some remaining debris which needs to be removed, add breeding water into the tank and then repeat step 3-1.
 - 3-3. Add new paramecia solution into the tank until 1.5 cm in depth.

Day 2

You should check the feed (paramecia) intake of larvae based on observing the following,

- What color is the abdomen (intestine) ?
 - if white, it's a sign that larva has eaten paramecia.
- Is the abdomen full?
 - if yes, it's a sign that larva has eaten enough paramecia.
- Is there any sign of feces?
 - if yes, it's a sign that larva has eaten paramecia.

If “no” in all questions, the number of paramecia in the tank is not enough. In this case, you should add more high-density solution of paramecia into the tank (There are two areas of high- and low-density paramecia within the culture solution.). From this day on, you should check those at least 1 hour after feeding until you can see any sign that the larvae have eaten paramecia.

4. At 9:00 (the time when you start work, the sooner the better), exchange breeding water according to step 3
5. At 16:00, exchange breeding water according to step 3

Day 3

The signs that the larvae are fed are probably observable.

6. Do steps 4 and 5

Days 4-7

Almost all of larvae can eat paramecia.

7. Do steps 4 and 5

Days 8-16

You should prepare brine shrimps and check the feed (brine shrimps) intake of larvae based on observing the following,

- What color is the abdomen (intestine) ?
 - if red, it's a sign that larva has eaten shrimps.

□ Is the abdomen full?

→ if yes, it's a sign that larva has eaten shrimps enough.

□ Is there any sign of feces?

→ if yes, it's a sign that larva has eaten shrimps.

From this day on, you should check those at least 1 hour after feeding until you can see the sign that larvae have eaten shrimps.

8. Repeat step 4

After that, add a small number of brine shrimps into the tank, and then observe larvae whether they are able catch them or not.

In the case that you want to breed fish of similar size,→If almost all of the larvae can eat them, exchange feeds for brine shrimp. If not, remove brine shrimp from the tank and try it again in the morning of the next day.

In the case that you don't care a variety of sizes in fish,→When you find the larva with orange abdomen (it's a sign that larva has eaten shrimps enough), move them to 3L-tank.

9. Repeat step 5

1st Day that almost all of larvae have eaten brine shrimp in the deep round container

Change breeding tank from deep round container to a 3L-tank. Be aware of phototaxis behavior of shrimps.

Brine shrimps have a positive phototaxis. In the case that a small part of the surface is exposed to light, brine shrimps will gather only in that area of the surface in the tank. If larva can't find shrimps in the case, you can create an environment, in which larvae can effectively find their feed by considering the light (e.g. the light is set in parallel with tank) and controlling the water depth (shown below).

10. Move larvae to 3L-tank by decanting

11. Place the tank in direction to the light source of the aquarium system to get an equal light distribution in the tank

12. Breed them under the condition of 2 cm in depth and standing water

13. At 1-2 hour after feeding, observe the feed intake of larvae. If you can see that the larvae are fed, increase breeding water up to 4-6 cm in depth.

From next day forward

14. Feed with shrimps and observe the feed intake of larvae. If you can see that the larvae are fed at the set water depth, add some more breeding water.

15. Repeat step 14 until the water depth has reached the required level for an aquarium with an overflow system.

16. Breed larvae as other types of medaka