

The method of preparing Paramecium

Paramecium sp. Y-2 (PS000001A) is larger paramecium species which is growing up infinitely with a culture medium described below. This strain is good food for medaka/zebrafish juvenile.

Materials

- 500 mL conical flask
- Breathable silicone stopper
- EBIOS (yeast extract-based nutrition mixture, Asahi Group Foods, Ltd.)
- Aluminum foil
- Sterile pipette for subculture
- About 10 mL primary culture solution of Paramecium (about 4-15 days after inoculation, photo 12-23)

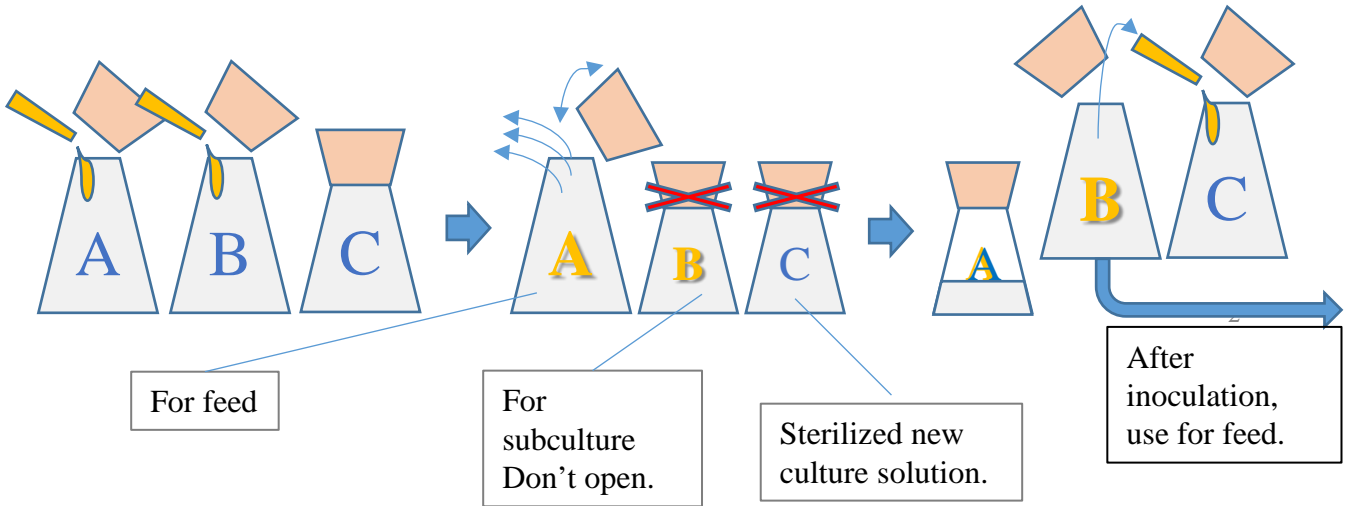
Paramecium for medaka/zebrafish food, Y-2 (PS000001A) strain, is available from NBRP Paramecium at Yamaguchi Univ.

http://nbrpcms.nig.ac.jp/paramecium/distribution_deposition/?lang=en

Preparation of culture solution

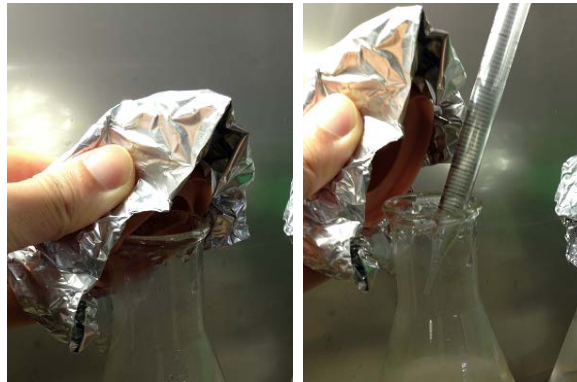
1. Add 450mL DDW and one tablet of EBIOS in 500 mL conical flask.
2. Be capped with a silicone plug to the flask, wrap the silicone plug with aluminum foil.
3. Sterilize by autoclave
4. Cool to room temperature

Feed preparation and subculture



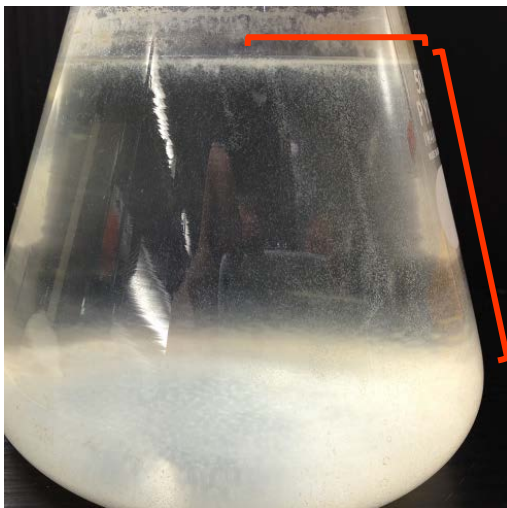
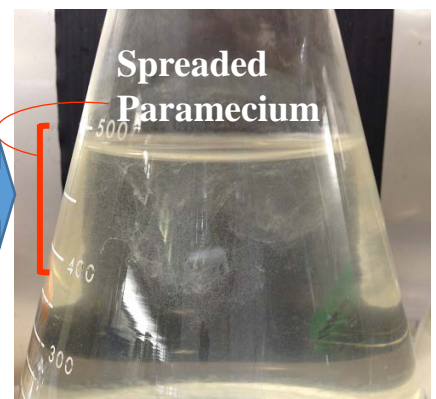
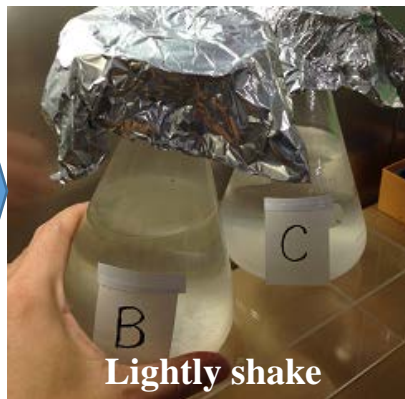
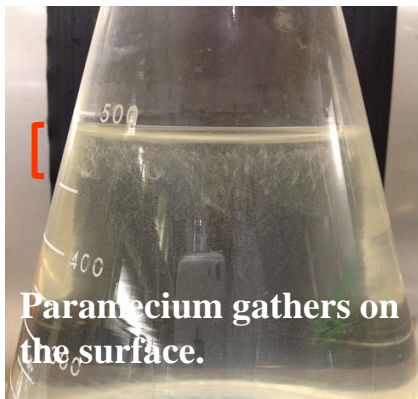
1. Prepare more than two conical flask with culture solution
2. Add about 10 mL primary culture solution of Paramecium using sterile pipette.
3. Static culture in the aquarium room for about 4-15 days. Don't open one flask before subculturing. Other flasks for feeds, pipettes that are not sterilized may be used.

*Silicone stopper is not fully opened for feed and subculture in the aquarium room.



4. Add about 10 ml add culture solution containing high-density Paramecium into new sterilized culture solution using sterile pipette.
5. Directly add culture solution containing high-density Paramecium into the tank for fry medaka fish. This solution doesn't have any lethal effect on larvae.

The red part is the region with high density of Paramecium.



Paramecium sometimes gathered on the side.



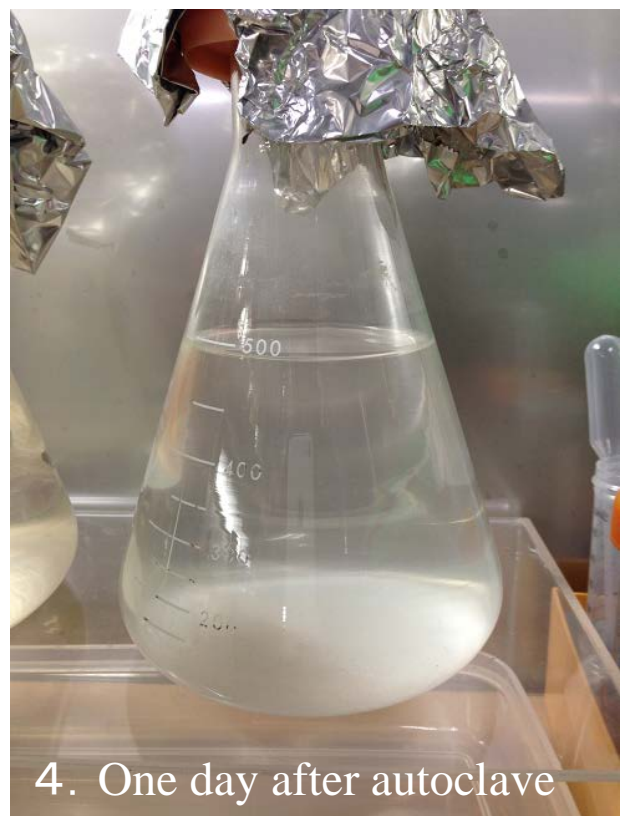
1. Before autoclave



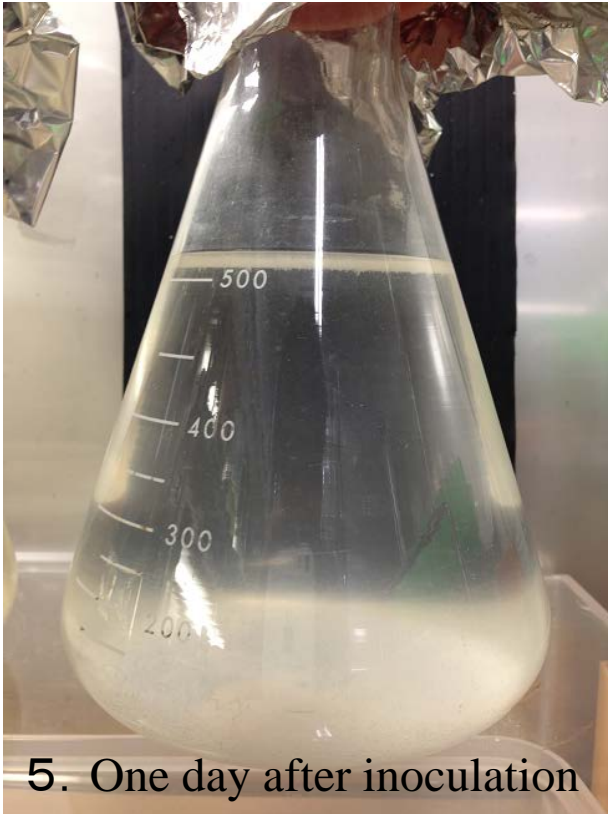
2. After autoclave



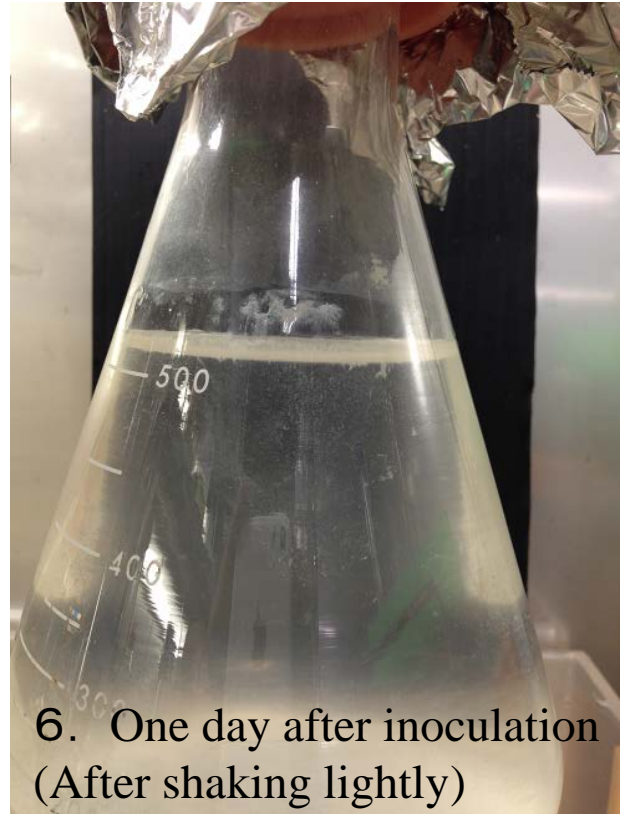
3. After autoclave



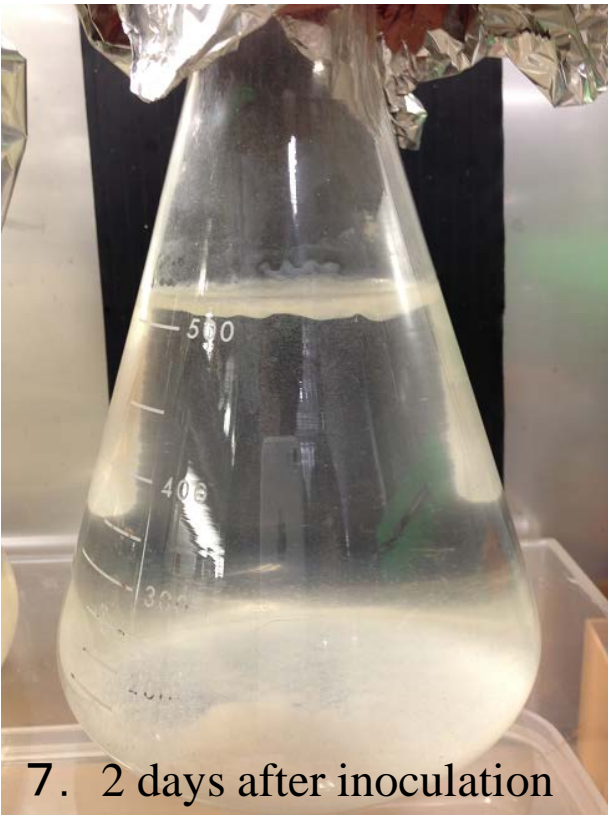
4. One day after autoclave



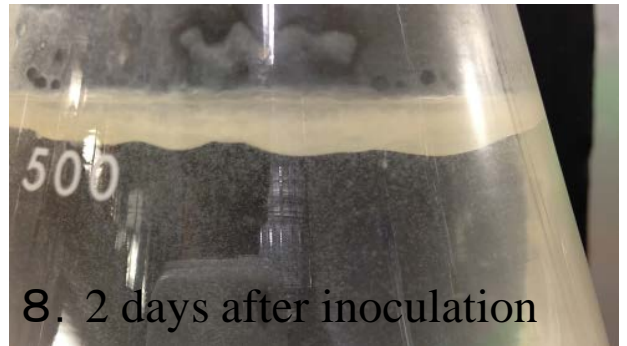
5. One day after inoculation



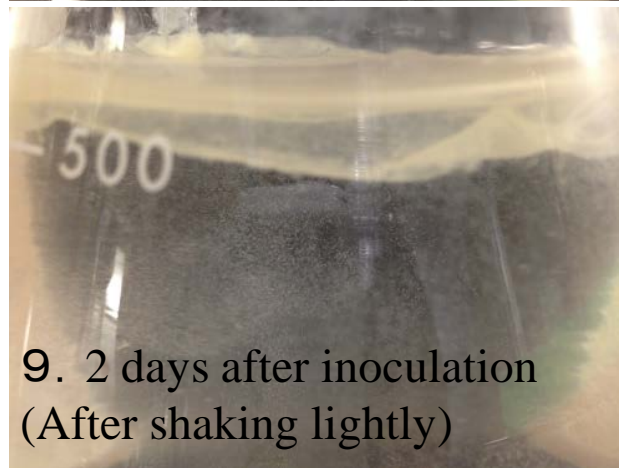
6. One day after inoculation
(After shaking lightly)



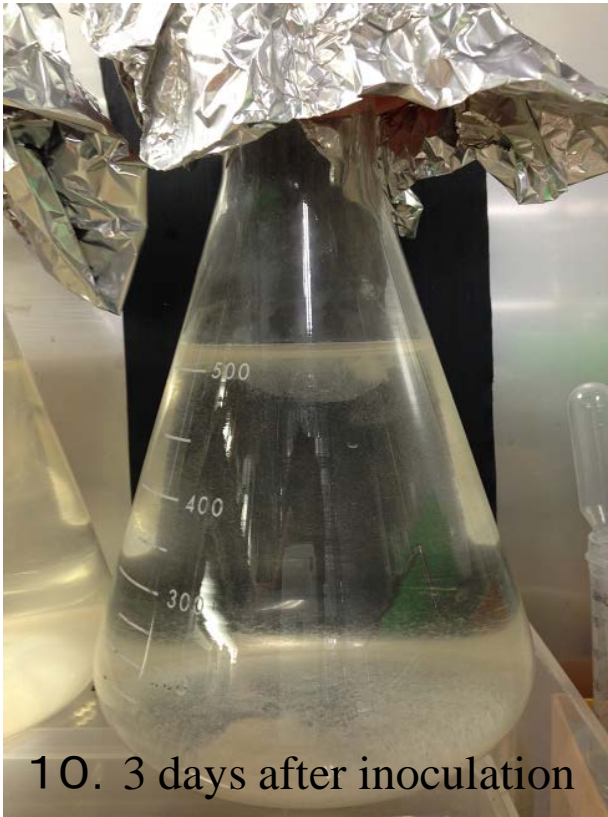
7. 2 days after inoculation



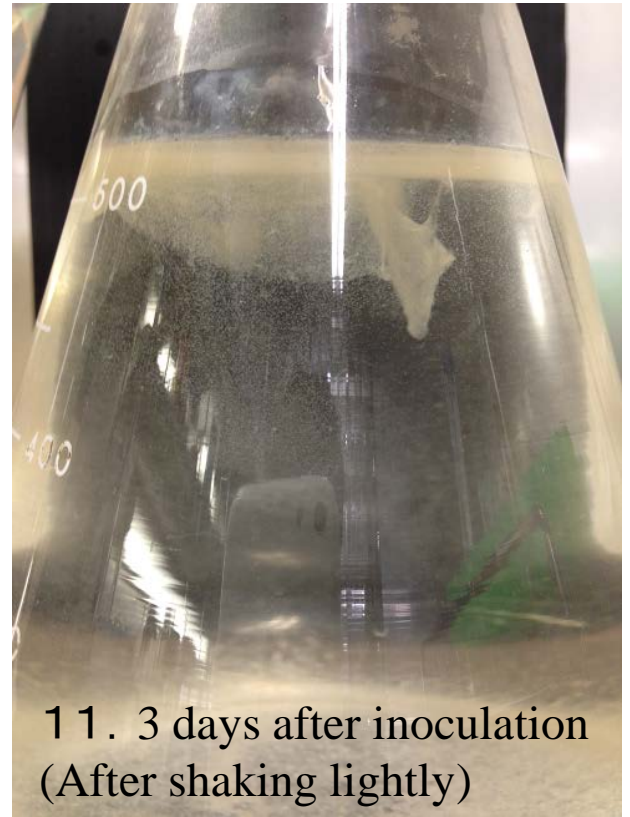
8. 2 days after inoculation



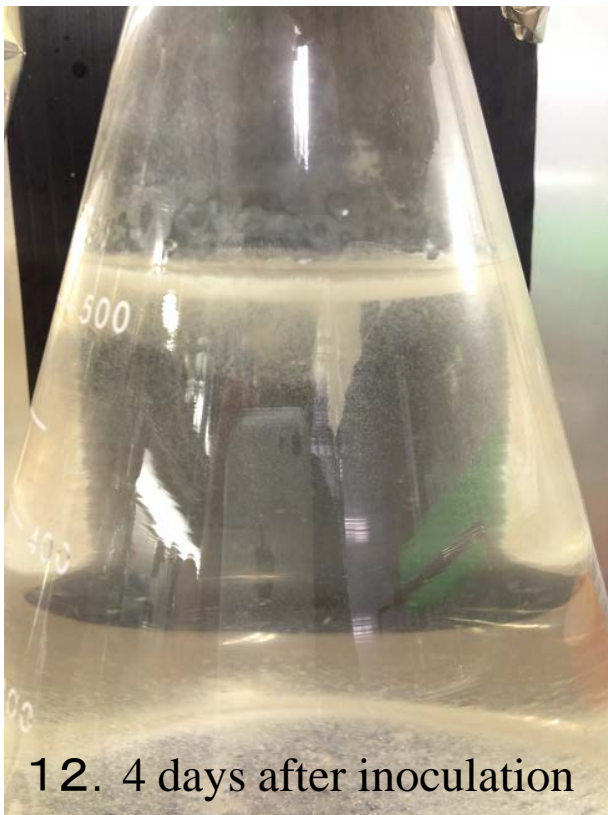
9. 2 days after inoculation
(After shaking lightly)



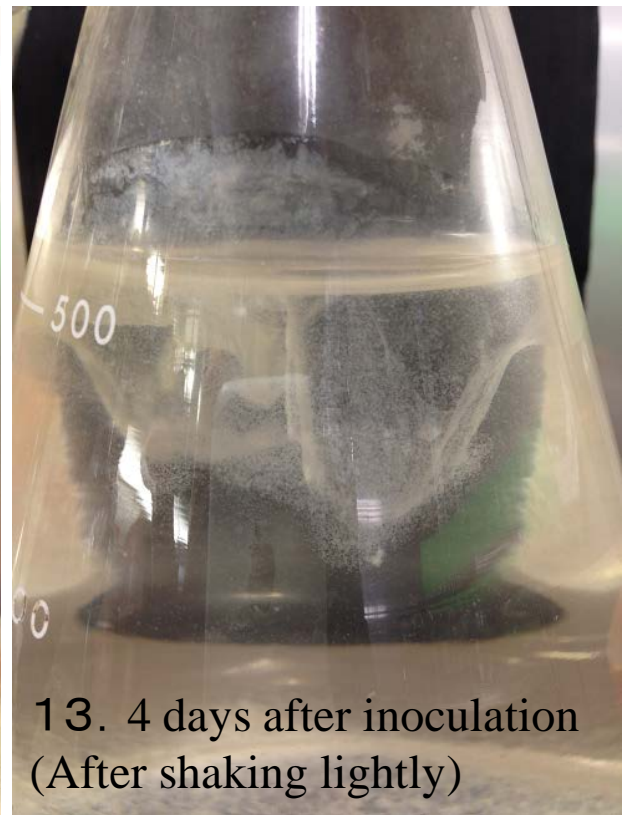
10. 3 days after inoculation



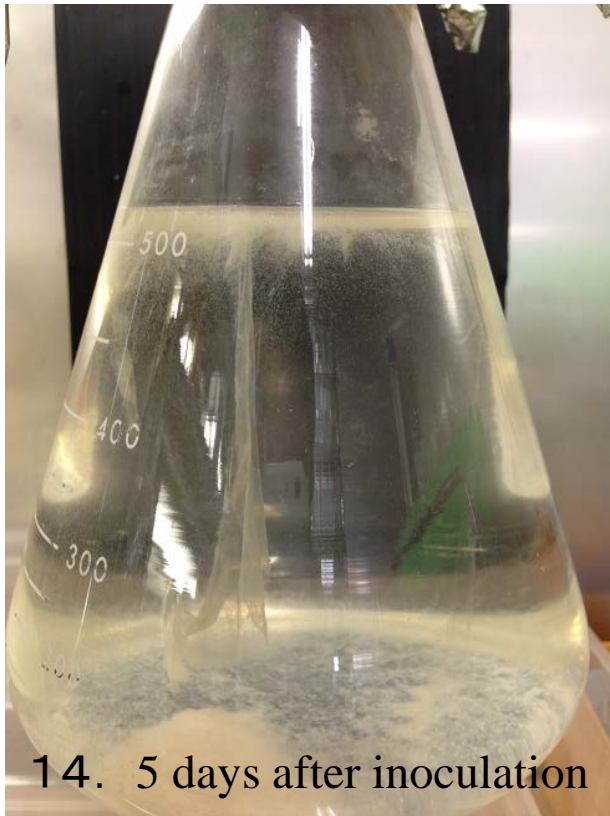
11. 3 days after inoculation
(After shaking lightly)



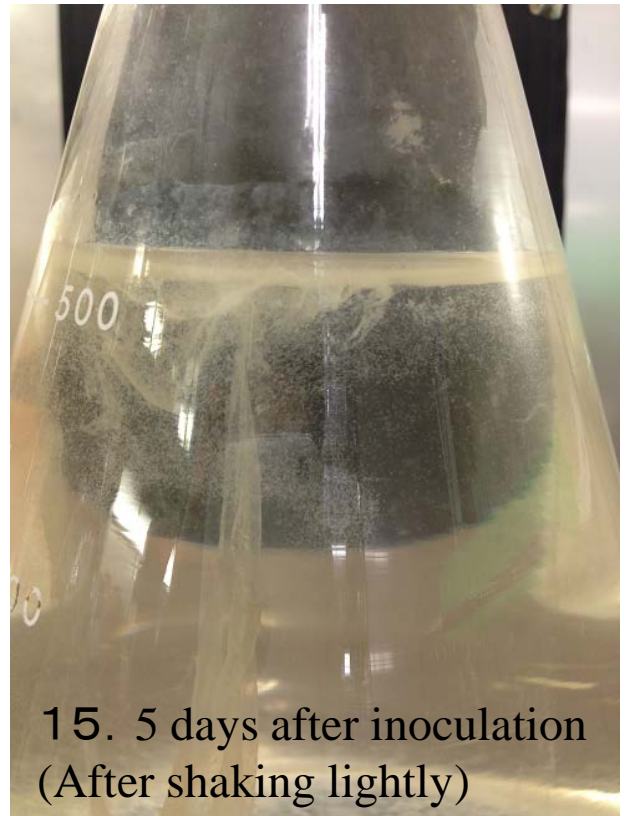
12. 4 days after inoculation



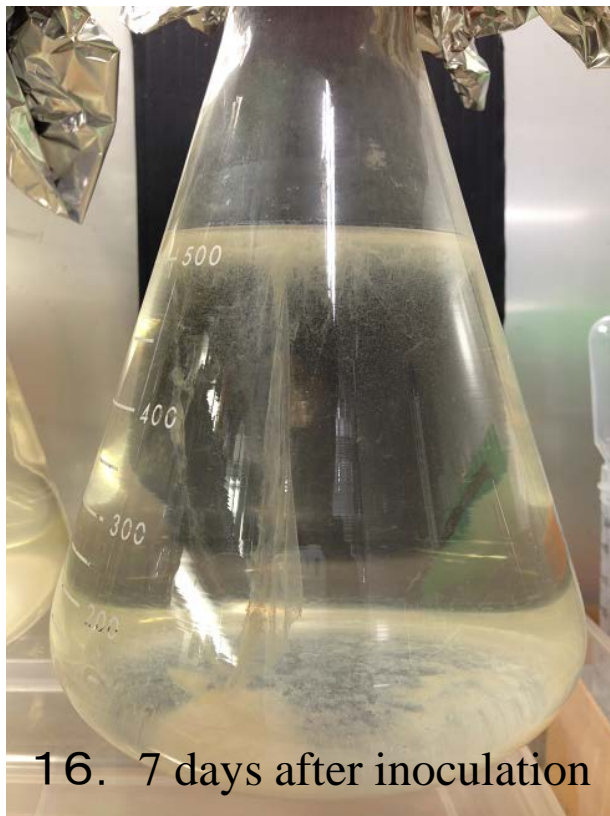
13. 4 days after inoculation
(After shaking lightly)



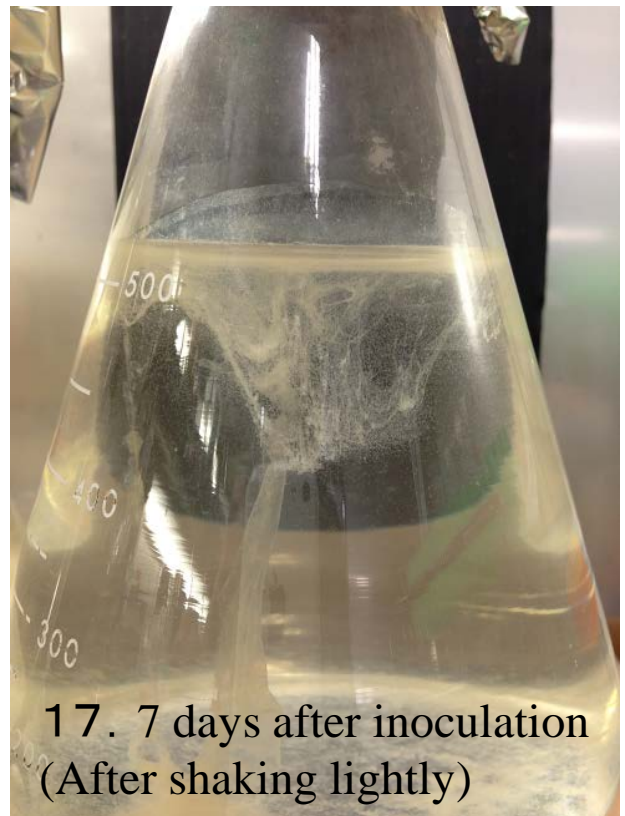
14. 5 days after inoculation



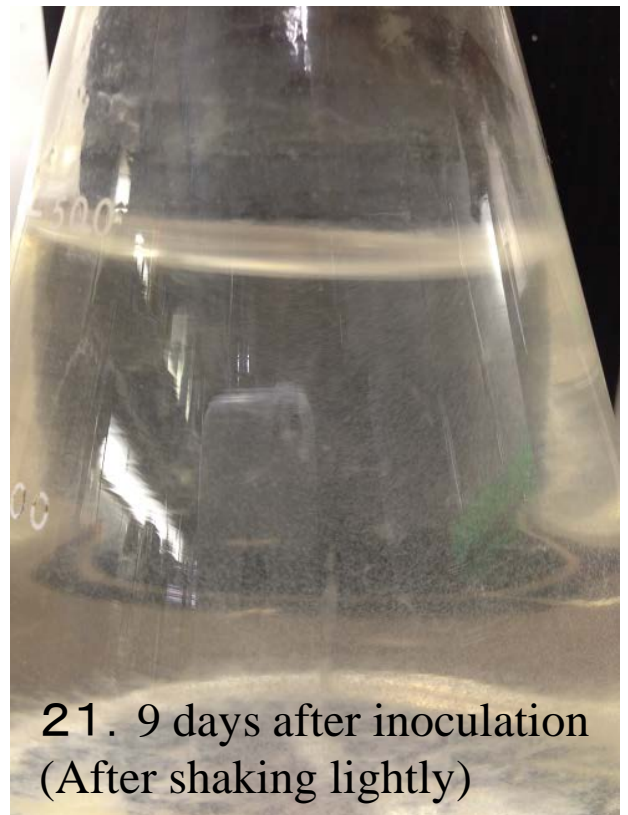
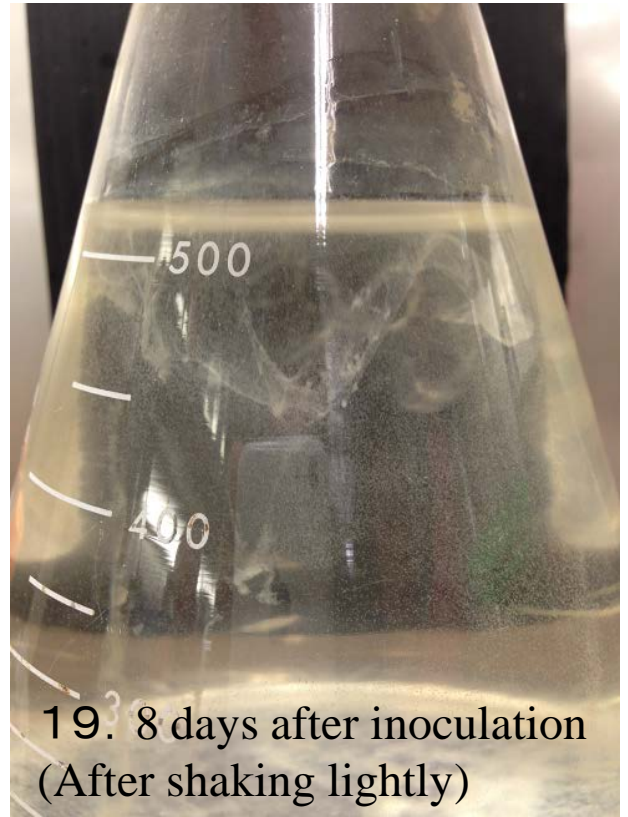
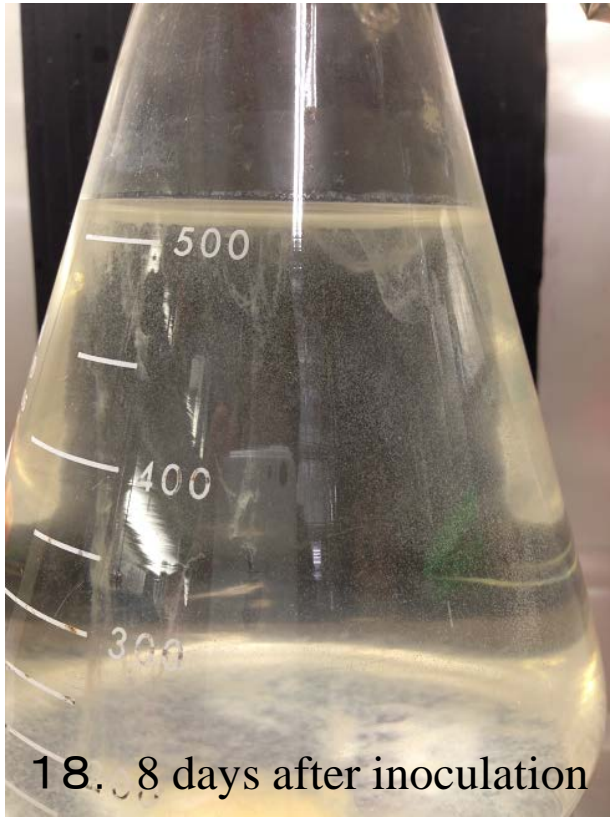
15. 5 days after inoculation
(After shaking lightly)

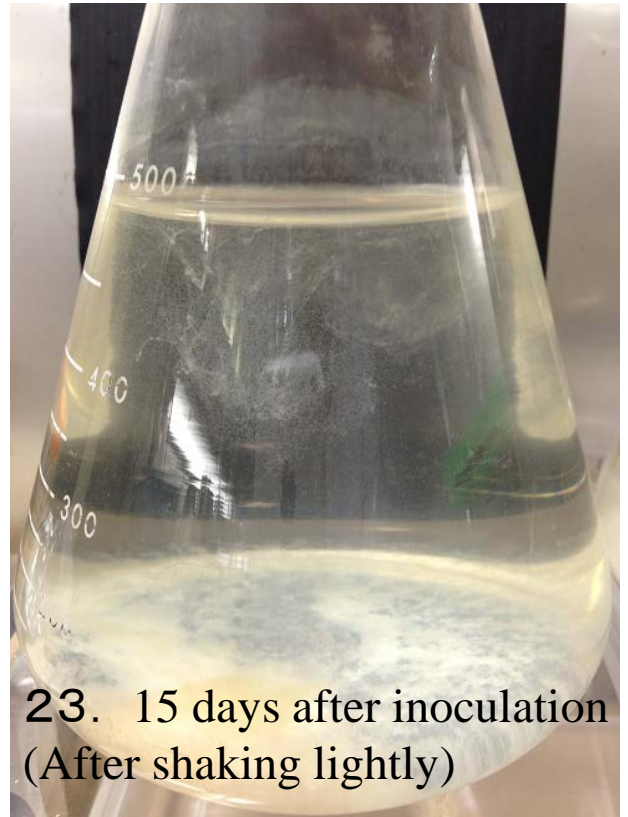
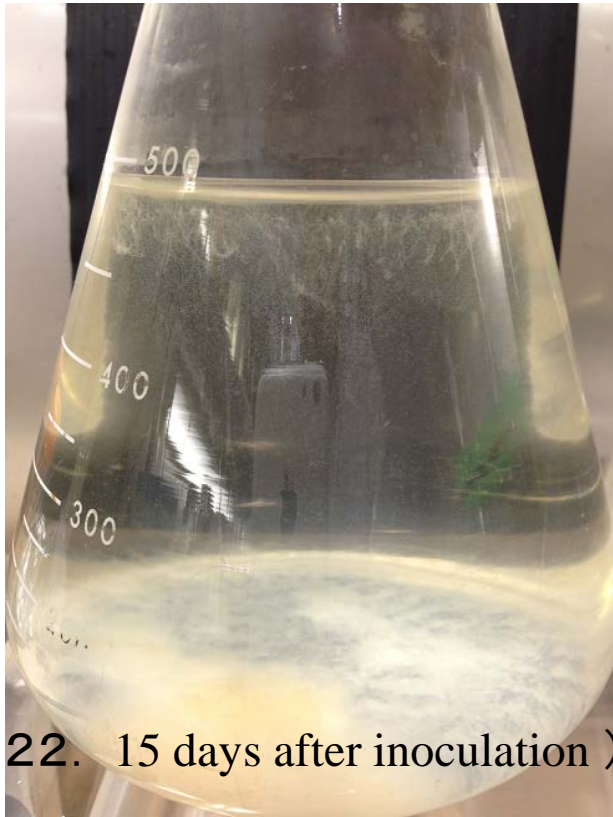


16. 7 days after inoculation



17. 7 days after inoculation
(After shaking lightly)



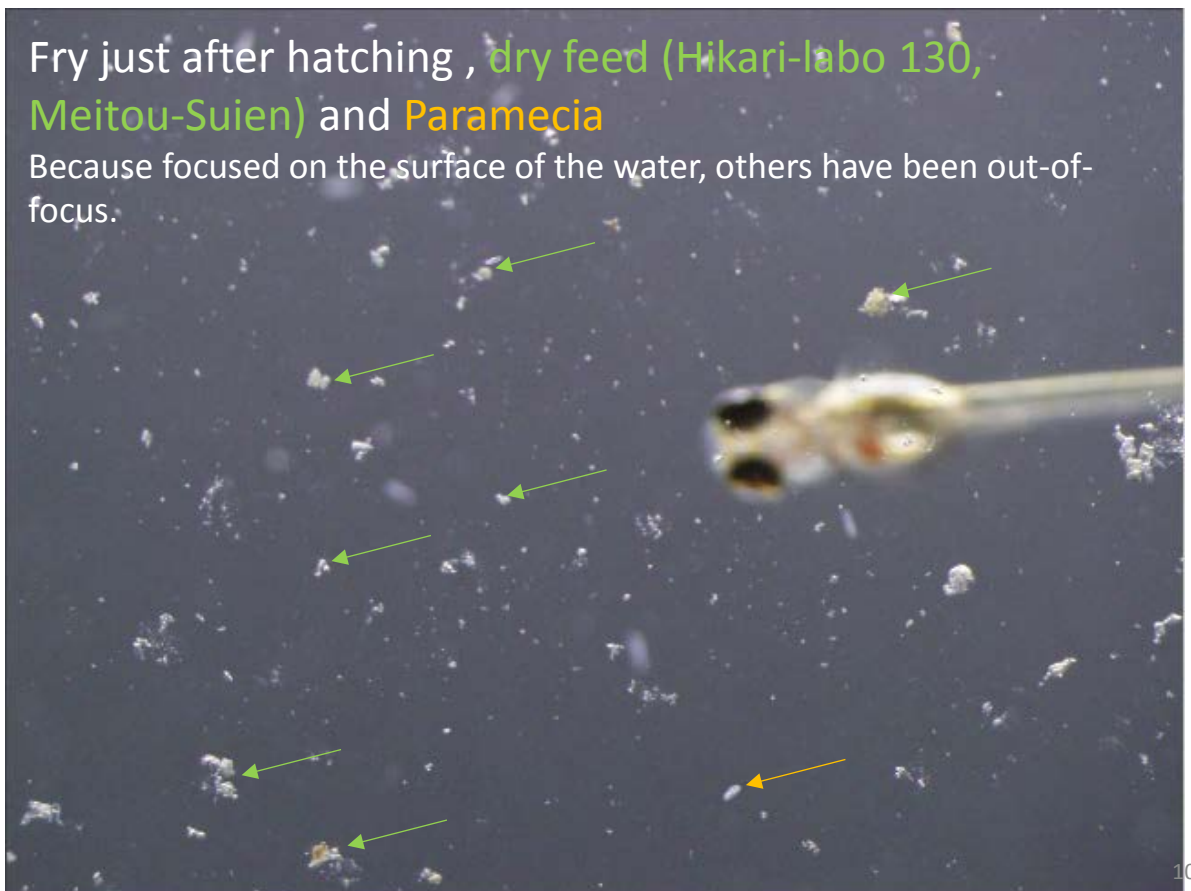


Fry just after hatching and **Paramecia**



Fry just after hatching , **dry feed (Hikari-labo 130, Meitou-Suien)** and **Paramecia**

Because focused on the surface of the water, others have been out-of-focus.





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