

Embryo and Larva Rearing

Rearing of embryos and larvae is one of our most important activities as it is essential for both normal stock maintenance and virtually all of our experiments.

Embryo rearing

- Shortly after breeding fish, embryos are sorted. Embryos must be sorted the same day they are bred. Only bother to sort as many as are needed and discard the remainder. Fertilized embryos that are developing normally are transferred by pipette to plastic dishes containing ~3 mm of embryo rearing water (system water, 10% Hank's solution, or the equivalent¹).
Incorrect media can kill embryos or cause abnormal development owing to pH or osmolarity that are either too high or too low, or because of trace chemical contamination. The amount of medium used is sufficient to dilute metabolic waste while still allowing for easy sorting under the microscope.
- For typical stock rearing, no more than 20–40 embryos should be counted into each dish. Under no circumstances should dishes hold more than 50 embryos as metabolic waste and low oxygen availability will adversely affect development and survivorship at higher densities. For rearing large numbers for in situs or other applications, up to 120 embryos can be counted into small rectangular breeding tanks.
For histological applications in which numerous embryos or larvae are needed, check with Dave to determine what housing conditions and densities are appropriate.
- Label every dish with a tank tag and record on it the number of embryos the dish contains (see **Stocks, Breeding Records, and Tank Tags SOP**).
- Add ONE drop of methylene blue stock solution¹.
Methylene blue inhibits fungal growth that can spread rapidly among embryos. However, it also can inhibit bacterial growth and is thus likely to be harmful to the fish system biological filter. We use it very sparingly.
- Inspect the embryos daily and remove any dead or dying individuals with a pipette; update the number of embryos on the tank tag to indicate stock health during these early stages.

Larval rearing

Static tank stage (~4 dpf to ~10–14 dpf)

- Once embryos have hatched, wash the embryos into a 2.8 L tank containing (from front to back): a 400 μm screen, a 1000 μm screen, and a solid baffle. Place the stock tag on the new tank. Add a single pink rotifer tag if ≤ 60 hatchlings; add two pink rotifer tags if > 60 hatchlings. Add larval water to the tank until it comes to the lower edge of the “window” on the screen baffle. Cover the tank and place in its FINAL location on the rack.

¹ See **Recipes SOP**.

Larval water is used instead of system water because the former has a higher salinity that prolongs rotifer survival. The amount of larval water in the tank and the time larvae are maintained without flowing water represents a compromise among several factors: less water keep encounter rates high for a given quantity of rotifers; it also keeps the surface to volume ratio high, which is important to allow gas exchange; on the other hand, less water means more concentrated waste products and less room for larvae. Note that you should not add additional methylene blue at this stage as it can kill the rotifers and inhibit normal development of gut bacteria. Enough will normally be transferred from the original dish to moderately inhibit fungal and bacterial growth, without other adverse consequences. If you are sorting embryos or hatchlings prior to placing them into a tank so that no methylene blue is transferred, add one drop of methylene blue to the tank at this time.

- Once larvae begin to swim (i.e., no longer adhere to the sides), they should start to be fed rotifers during daily fish maintenance.
Rotifers should not be fed too soon, as uneaten rotifers will foul the water. Nor should they be fed too late, as starved fish are unlikely to fully recover. We begin feeding in a relatively small volume to maintain an initially high rotifer density and encounter rate without having to add excessive numbers of rotifers that will only die and add to the crud at the bottom.
- Monitor the larvae while they are in the static tank stage to make sure that they are growing and healthy, and that the water remains clear.
If you observe mortality during this time, you may need to discard the fish and rear replacements (see Stock Maintenance SOP). If the water becomes cloudy, this indicates excess bacterial growth; depending on severity of the problem, you may need to turn the water on prematurely or discard the fish.
- Once larvae are placed on the rack, changes in feeding and water flow (below) are performed as part of routine maintenance. Nevertheless, you should check your fish regularly to make sure they are doing ok and progressing through the normal sequence of care regimens described below: if you bring fish into this world, you are responsible for ensuring their continued care and well-being, including culling or splitting, as required. If your fish have special needs (e.g., prolonged rotifer feeding or different water salinity) be sure to check with Dave, and then label the tank accordingly.

Low flow tank stage (~10–14 dpf to ~28 dpf)

- After several days in a static tank, larvae will be eating rotifers efficiently and will have grown large enough to eat juvenile food (typically, ~11 dpf). If larvae are large enough, the system water is turned on to a low but constant flow and a yellow food tag should be affixed to the tank so the larvae begin receiving juvenile food.
***Important:** If larvae are growing more slowly than normal, either because of genetic background or if rotifers are limited, it is better to keep them in a static tank than to turn on their water prematurely. Since ChlorAm-X/bicarb is provided to static tanks with the rotifers (see **HD Rotifers SOP**), the risk of ammonia and pH toxicity is minimal compared to the risk of fish starving because they are too small to efficiently find food in a tank with flowing water. If the rotifer supply is*

low, keeping fish in static tanks will also help to conserve rotifers as static tanks require fewer rotifers than flowing tanks.

- The larvae will be transitioned off of rotifers and onto a mixture of juvenile and adult dry food after several additional days, with the precise time depending on growth rate.
- After a few of days on flowing water, the 400 μm screen will start to clog and should be removed from the tank to facilitate water flow. Even if clean, this smaller screen should be removed as soon as larvae are large enough to be confined by the larger 1000 μm screen.
- Occasionally, small larvae will need to be transferred to new tanks. To make the transfer, get a new, clean tank and insert a solid baffle in the back position and a 1000 μm screen in front of it. Fill with a couple inches of system water. Place a large blue-handled, fine-mesh white net just above another tank filled with system water. Pour the contents of the original tank through the net. Then, gently lift the larvae out of the water and transfer them to the new tank.
This particular net works well for straining out detritus from the tank while retaining the larvae themselves. Placing the net over water allows the delicate larvae to stay mostly immersed, thereby preventing injury.

High flow tank stage (~28+ dpf)

- When fish have reached the juvenile stage and cannot escape beneath the solid baffle, they are transferred to a clean tank with only the solid baffle and no screen. At this time, water is turned up to a higher flow rate, as specified in the **Fish Maintenance SOP**.

