

Protocol for Raising Zebrafish Embryos (0-30 dpf)

Immature zebrafish require intensive care during the first few weeks of life. To ensure that they grow into healthy, productive adults, we follow the following protocol. This is a modified version of the Weissman Lab protocol and developed based on the nursery procedure used by the Zebrafish International Resource Center (ZIRC).

Quick Guides for 0 – 21 dpf

	0 -4 dpf	5 -9 dpf	10-11 dpf	12-14 dpf	14-17 dpf	18-20 dpf	21 dpf
Daily feedings	None	1.HEP 2.AP 3.HEP (3times a day)	AM: AP+HEP PM: AP+HEP (2 times a day)	13 dpf: Starting to check if they can eat shrimp	(keep shrimp checking)	AM: AP+shrimp PM: AP+shrimp By 18-20 dpf, all fish should eat shrimp	AM: Shrimp+powder PM: Shrimp+powder
Tanks and cleaning	Prtri dish (<100 eggs/dish) in the small incubator	Small tanks with FW line placed in the nursing tank	5-21 dpf: You need to remove all floating stuff and clean the bottom of the tank				Mesh tanks in glass tanks or small tanks with a fine mesh hole in slow water dripping tank
Water	EM	5 dpf: Original ME+FW to ~1cm	6-12 dpf: Add 1cm FW(125ml)+ the amount of FW you take out during cleaning.	Once FW levels reach the FWline replace 150 ml FW+ the amount of FW taken out during cleaning each day			Transfer to new tanks
others	Incubator temp 28C	5 dpf: Transfer to the nursing tank Clean tanks and add or replace FW before feeding.					21 dpf: Transfer to the regular tanks system After 21 dpf, a person who are in charge for fish care and feeding takes care of fish Label your tank clearly that this is a new baby tank and no flakes

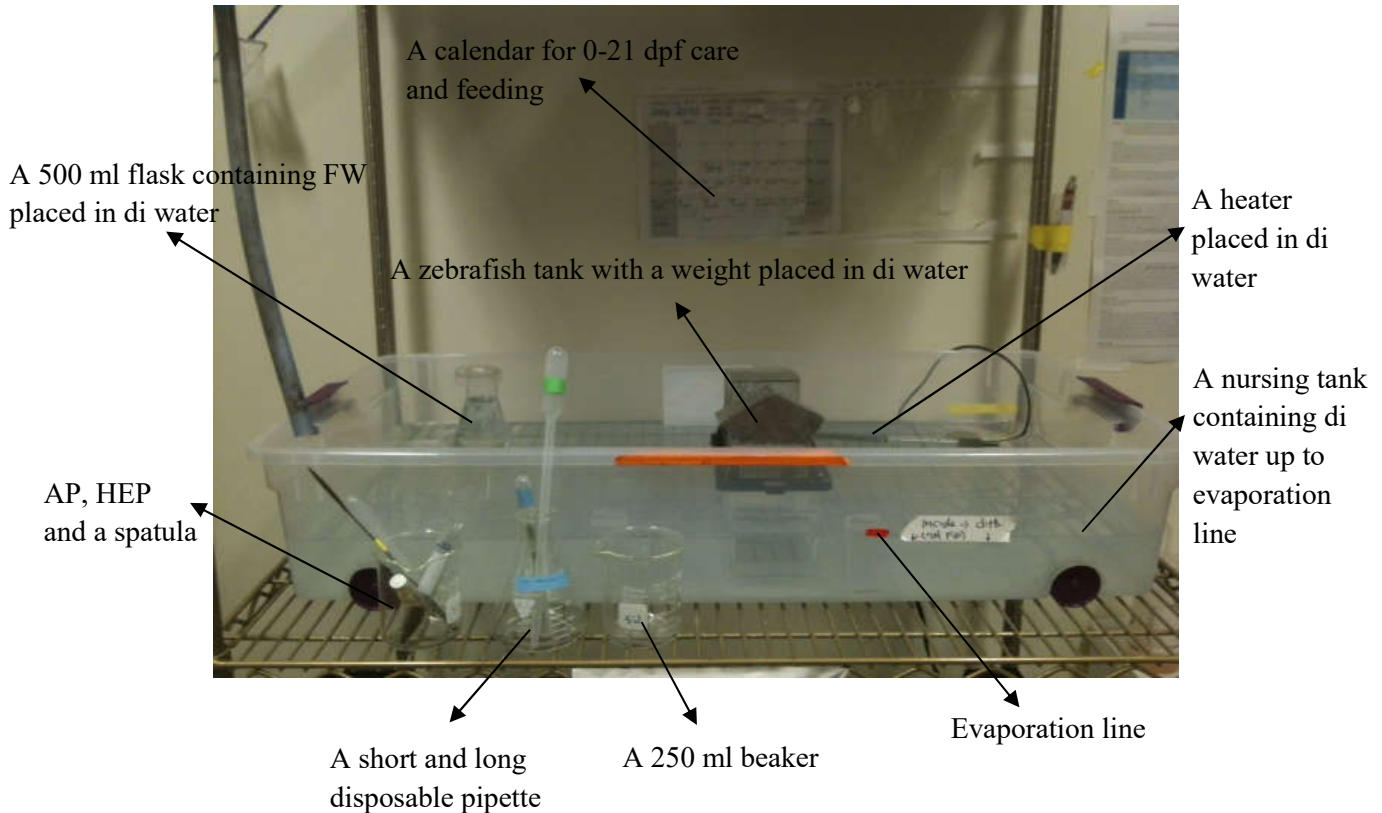
- Note:** - HEP: Hatchfry encapsulation powder (orange); AP: Zeigler AP 100 powder (Green)
 - Write daily logs including group codes, dof, doh, # of fish in each tank, # of fish died, the day you transfer tanks, the day they start eating shrimp and etc.
 - **Clean around the sink every time you feed. Make sure that you do not leave anything there.**

Day 0-4 (in a small incubator at lab 277)

Carefully collect eggs and rinse them with EM to remove debris. Eggs are kept in 9 cm petri-dishes at a density of <100 eggs. Label each petri-dish and start writing daily logs. Be sure to remove any empty choria and dead eggs/embryos from the dish every day, as they will foul the water. Replace 3-4 pipettes of EM every day. At this time, **feeding is not necessary**. Set the Petri dishes in the incubator with 26-28 C. Prepare a small larval tanks with a top water level line (about 5 cm below the lid), and set the nursing tank containing warm di water

up to the evaporation line, a 500 ml flask with FW, a heater and a thermometer in the animal room by 5 dpf (see the below picture)

Nursing tank set up and materials you need



Day 5

Transfer the fish (with their EM) into the small larval tanks at a density of <100 fish per tank and add a small amount of regular fish water (FW) to achieve a depth of 1 cm. **Transfer the tanks in the large nursing tank** in the animal room 1. Carefully place two weights on the lid of tank so that the tank won't be flipped over.

Day 5-9

Around 5 dpf, the yolks will become depleted and feeding will become absolutely necessary. Starting on Day 5, give the fish a miniscule amount (like, as little as you can manage) of powder with a spatula **three times per day (AM, noon and PM)**; overfeeding will foul the water. Give AP (Zeigler Larval AP100 powder, Green color) at AM and PM, and HEP (Hatchfry Encapsulation Powder, Orange color) around the noon. Adjust amount of powder while they are growing. **Cleaning tanks are very important**; clean tanks before feeding 3 times a day. Carefully remove the weights while holding the tanks, and place the tanks near the sink. Using pipettes and a beaker, remove all floating stuff and debris & dead fish in the bottom of each tank into the beaker. Do not dump dirty water directly into the sink (You need to know how much FW you take out). If the leftover powder foods are floating, fish won't come up to eat and they will die. Check the beaker if any zebrafish are there before dumping dirty FW out. During this period, **gradually increase the water level** in the larval tanks by adding **1 cm of warm FW (125 ml) + amount of FW you take out during cleaning every day** (NOTE: adding 125 ml FW is once a day, but you replace FW taken out with a new FW every time before you feed).

Days 10 & 11

Increase food amount by giving both HEP and AP in the AM and PM feedings (2 times a day). The most larvae prefer AP so that give AP mainly and add small amounts of HEP. At this time, you should see, all fish come up to the surface when you feed. If the powdered food appears to be eaten very rapidly, you should increase the amount, until most is gone within 5 min. Keep doing 1 cm FW increment each day until FW level reaches the marked top level line (approximately 5 cm below the top edge of tank).

Days 12-15

FW level should reach the marked top line during this time period. Now, replace 125 ml FW+ amount of FW you remove during cleaning every day. To check if they can eat shrimp, add small amount of brine shrimp to the HEP/AP regimen around 13~14 dpf. If they don't eat yet, remove shrimp from the tanks. You can easily see it as the color of zebrafish turns to orange when they eat shrimp. Once a majority of fish starts eating shrimp adjust feedings so that most of the shrimp are eaten within about 5 min. Again, if the food appears to be eaten very rapidly, you should increase the amount, until most is gone within 5 min. We want food to be abundantly available so that the growing fish develop rapidly and evenly (but not too much food). You may begin to notice a disparity in the size of your fish. This can indicate that you are not providing enough food and the fish are competing for what is available. The 15-day marks an important milestone; If your fish survive and appear healthy beyond this point, you have done a good job.

Check list for daily care

1. Add di water up to the evaporation line in the nursing tank (if water level is too low, the heater starts burning)
2. Carefully remove the zebrafish tank and the 500 ml flask with warm FW from the nursing tank and place them near the sink.
3. Do cleaning and FW adding (once a day) and replacement (every time you clean).
4. Check the beaker with dirty FW before dumping. Sometimes you accidentally pick up larvae while cleaning.
5. Feed fish.
6. Repeat 3-5 for each tank.
7. Put back the zebrafish tank with weights in the nursing tank.
8. Put fresh FW into the 500 ml flask and place it back in the nursing tank for the next use
9. Clean around the sink (We share this room with other labs. Do not leave anything near the sink).

Days 18-20

Maintain the same feeding regimen (2X per day, brine shrimp plus HEP or AP) and water replacement. Check if all fish can eat shrimp. Once they start eating shrimp, they grow rapidly

Day 21

It's graduation day! By now, all your fish should eat shrimp and be big enough to **transfer to the new tank system**. Label a new small tank with a fine mesh hale, place a filter (screen) pad at the back of a mesh hale before you transfer. Fish should be <20/per small tank and ensure fish are big enough not to escape through mesh hale. Alternatively, transfer fish to a mesh tank (use two double mesh sheets) and place it in the zebrafish glass tank. In some instances, 21-day-old fish are still not big enough for transferring; if yours still look a little small, keep caring for them as you have been until they get a bit bigger. In the new tank system, clearly label tanks so that people who take care of fish can know that these tanks are for new babies. Put sign of "no flakes" in each tank and put notes indicating use a small pipette and a beaker for cleaning. Ensure that the water flow is active, but slow dripping. Email or tell the fish care people directly that you transferred the new baby fish and ask special attention for them.

Day 30

Check if they are full grown and can eat flaks. If they can eat flakes, remove the sign and the notes.