**STANDARD OPERATING PROCEDURES - REARING OF ZEBRAFISH EMBRYOS**

**Preparing embryos for the nursery**

1. Prepare fish crosses on Tuesday to collect embryos on Wednesday.
2. Make a solution of bleach water by placing 0.1 ml of 5.25% sodium hypochlorite (bleach) and 170 ml of system water into a 250 ml beaker.
3. Rinse embryos (6 to 24 hours old) in Ringer’s solution (embryo media) and then place 25 to 30 into the beaker of bleach solution.
4. Swirl the beaker and allow the eggs to separate and the entire surface of each chorion to come into contact with the water.
5. After 2 minutes in the solution, remove the eggs with a clean pipette and place them into a beaker of clean water.
6. Rinse embryos thoroughly by swirl the eggs in clean water.
7. Repeat the rinse procedure 2 more times.
8. Place 60 embryos in a Petri dish with embryo media. Prepare as many Petri dishes as needed. Place dishes in 28°C incubator in fish facility.
9. A tough chorion can often be a by-product of the bleaching procedure.  Thus, if the young fish do not hatch on their own by day 3 or 4, remove the chorions manually.
10. On Monday, 5 days after egg laying, transfer embryos a small crossing tank. Feed with enough food to fill the tip of a Pasteur pipete (Zeiglers AP100). After 10 minutes, fill tank ¾ of the way with embryo media and place in water bath at 28°C.

**Week one**

1. Morning.
   1. Feed larvae with enough food to fill the tip of a Pasteur pipette (Zeiglers AP100).
   2. After 10-20 minutes, clean bottom of tank with Pasteur pipette or small piece of plastic tubing working as a syphon. Decant some of the liquid to remove food floating on the surface of water. Remove ½ volume of liquid and replace with fresh embryo media.
2. Noon
   1. Feed larvae with enough food to fill the tip of a Pasteur pipette (Zeiglers AP100).
3. Late afternoon
   1. Feed larvae with enough food to fill the tip of a Pasteur pipette (Zeiglers AP100).
   2. Check for and remove dead larvae.

**Week two**

1. Follow instructions for week one with following modifications
   1. Instead of using embryo media for water exchanges, use facility water that has been filtered through a 0.22 micron filter.

**Week three on**

1. Gently transfer juveniles to a fish facility tank that has a small mesh backing. Place in recirculating system with a low water flow.
2. Continue feeding three times a day. As fish start growing in size, replace the food for increased particle size (Zeiglers AP150-200>Zeiglers AP200-250>Adult food).
3. When juveniles reach appropriate size, supplement diet with live brine shrimps.