

University of Oregon
Zebrafish International Resource Center

TITLE: Larval Rearing Procedure

SUBJECT AREA: Zebrafish Husbandry and Line Maintenance

PROCEDURES:

Summary:

Housing: A variety of commercial nursery systems are available through zebrafish rack manufacturers and can be found on-line or by contacting zirc@zebrafish.org for more information. Additionally, some facilities have developed their own nursery systems using materials readily available at home improvement stores for a fraction of the cost of a commercial unit.

Alternatively, larval care can be accomplished by housing the fish in small containers of water with no flow (static conditions). This method will require daily hand siphoning to remove debris, manual water changes and frequent monitoring of water quality conditions (ammonia, pH, etc.).

Multiple nurseries. Due to increased biosecurity concerns throughout the research community, it may be necessary to have separate nurseries on separate water systems. This gives more flexibility and security when dealing with fish that have or may have a questionable health status, whether from an in-house line or a new import into a quarantine room.

Schedule overview. To simplify the nursery schedule and avoid complicated weekend care, all fish at ZIRC are born Sunday-Wednesday of each week, with no fish born Thursday thru Saturday. Every Monday, 5-8 day post fertilization (dpf) larval fish (born the previous week) are transferred from Petri dishes to nursery tanks with no flowing water. Soon after this transfer, the fish are provided their first meal of paramecia, which they will receive daily for 5 days (Monday thru Friday). On Saturday of each week, the paramecia feedings are suspended, the fish are placed on running water, and the feedings are changed to Larval Mix and artemia. See Procedure Overview below.

Water supply. To provide an optimal rearing environment, the ability to control water quality by adjusting water flow to each nursery tank, according to the specific requirements of the growing larvae (e.g. mouth gape), is key. This requires that tanks have individual water valves and drains that allow for switching from static water conditions (water off) to intermittent or constant 24-hour water drip or flow. The ZIRC nursery racks are tied into the facility's centralized recirculating water systems, with a nursery rack on each of the 2 systems to maintain biosafety.

During the first week of life, embryos and young larvae at ZIRC are maintained at a density of 50 fish per Petri dish, at 28°C in 0.5x E2 medium and methylene blue (0.5

ppm) for 5 to 8 days post fertilization. On Mondays, 5-8-day post fertilization (dpf) larvae are transferred to nursery tanks that contains a small amount of fish system water (approximately 150 ml/tank). Larvae are maintained in static water for 5 days (Monday thru Friday) and only water containing clean paramecia is added during this time. At 10-12 dpf (each Saturday), water is turned on to each tank at low flow and remains on permanently.

Food. While in the nursery, the larvae are given different types of food that increase in size as the fish grow and their gape increases. Paramecia are introduced at 5-8 dpf and fed through day 8-12 dpf. A powder diet consisting of equal parts Zeigler AP 100 and freeze-dried rotifers (Larval Mix) is introduced on days 10 to 12 after tanks receive 24-hour water flow. San Francisco Bay Artemia (brine shrimp) are introduced concurrently to the Larval Mix and used for the remainder of time in the nursery. See Fish Feeding for live and dry food recipes (<https://zebrafish.org/wiki/protocols/nursery>).

Note: The first 5 days of feeding is critical. If the larval fish do not receive enough food during this period, high rates of mortality can occur between the age of 11-15 day post fertilization. Maintaining ideal water quality conditions during this time is also important for good survival.

Since 2013 ZIRC has been experimenting with the use of freshwater rotifers as a live feed during the static phase in the nursery when nursery submissions exceed 3500 fish. Since we find them useful for busy weeks, rather than culturing yet another live feed in-house, we have elected to order them when needed from Reed Mariculture. Before rotifers can be fed to the fish, they must go through bio-safety testing by the ZIRC veterinarian. Although supplementing the nursery with rotifers is rare, it has been helpful for taking the pressure off of our paramecia colony. We have chosen not to change the conductivity of the water for rotifers; instead they are treated like brine shrimp - strained, rinsed, and then fed two times a day in the static phase.

Juvenile (a.k.a. Grow-out) section. After larvae have been in the nursery for 25 days, they are transferred to the juvenile (a.k.a. grow-out) section and into adult tanks with fine mesh drains. During this time they are fed a special juvenile diet (2 feedings per day). Developing fish are maintained in the juvenile section until they have reached adult size, typically around three months of age.

Statistics. Embryos and larvae are sorted into Petri dishes at a density of 50 embryos per dish. At 5 days, nursery staff remove undeveloped, dead, dying, or mutant larvae, and record the number of fish per stock going into the nursery (the baseline for nursery statistics). At 25 days, the surviving juvenile fish are counted again as they are transferred from the nursery to the juvenile section. Mortality rates are generally very low, however when assessing mortality rates, we consider changes in the wild-type background as well as characteristics of particular lines (mutations, transgenes etc.).

Procedure Overview:

Feeding

- 5 to 11 dpf (days post-fertilization) - fish are fed concentrated paramecia and occasionally rotifers.
- 12 to 25 dpf - fish are fed Larval Mix and artemia, both 2x per day

Water Changes

- 0 to 5-8 dpf - fish are kept in Petri dishes with E2 embryo medium
- 5 to 8 dpf (every Monday) - fish are transferred to nursery tanks (static system water)
- 5 to 11 dpf - fish are maintained in static system water
- 12+ dpf - fish receive 24-hour water flow (constant)

Detailed Nursery Procedures:

Day 0 to 5-8:

- Maintain embryos/larvae at 28.5 °C in Petri dishes (100 X 20mm) with E2 embryo medium at a density of 50 fish per dish

Day 5-8 (every Monday):

- Transfer larvae to nursery tanks, with 150 ml of system water

Day 5 - 11:

- Maintain larvae in static system water
- Each morning, feed 10 ml concentrated paramecia and/or rotifers
- Every afternoon, feed 10 ml concentrated paramecia and/or rotifers

Day 12 (Saturday of each week):

- Discontinue paramecia/rotifer feedings
- Switch to constant (24-hour) water flow
- Feed larvae 5 ml Larval Mix (powdered food prepared from Zeigler and Freeze-Dried Rotifers), twice per day
- Feed 5 ml concentrated artemia, twice per day

Day 12 - 25:

- Continue feeding Larval Mix and artemia, each twice per day
- Water flows 24-hours a day

Day 25-30:

- Juvenile fish are usually ready to be moved from the nursery to the juvenile (a.k.a. grow-out) section of the main facility
- Continue nursery care if larvae are not large enough to be transferred out of the nursery
- Recount and transfer the juvenile fish from the nursery to 1-gallon (3.75 liter) tanks and place them in the juvenile section. To prevent fish from escaping, fine mesh juvenile drains are required.

Day 25 - 80:

- Feed artemia and a juvenile powder mix (total of 2 feedings per day)
- Once the fish are large enough (2 - 3 months of age), mesh drains can be replaced with adult drains and adult master mix fed to them. The fish can now be moved from the juvenile section to the adult sections.

Stage definitions for Embryo, Larvae, Juvenile:

Parichy, D.M., Elizondo, M.R., Mills, M.G., Gordon, T.N., and Engeszer, R.E. (2009) Normal table of postembryonic zebrafish development: Staging by externally visible anatomy of the living fish. *Dev. Dyn.* 238(12): 2975-3015.