

Standard Operating Procedures

Chen Laboratory Zebrafish Facility: CCSR 3152

General Rules

1. Before and after handling fish or system water, hands must be washed with soap and water.
2. Water and fish from the Quarantine system (*indicated with YELLOW labels*) must never be introduced to the Main system (*indicated with WHITE labels*).
3. Separate fish nets should be used for the Quarantine and Main systems. *Green fish nets* are used for the Quarantine system, and *white fish nets* are reserved for the Main system. The nets should be sanitized in Net Soak-containing solutions in between uses.
4. Fish should only be exposed to system water as tap water contains chlorine or chloramines, which kills fish.
5. Watch for signs of overfeeding: fat fish, uneaten food on the bottom of the tank, clogged screens in baby tanks. The rule of thumb is to give the fish as much as they will eat in about 5 minutes.
6. Watch for signs of underfeeding: skinny fish, really hungry fish, slow growth of young fish.
7. Do not enter the fish room when the lights are out (between 11 PM and 9 AM).

Fish Health

1. Remove any dead fish or fish that display symptoms of disease, and record these data in the fish facility log. If pathology services are warranted, sick fish should be kept alive and shipped to either the Stanford Dept. of Comparative Medicine staff or the Zebrafish International Resource Center's pathology services. Dead fish should be frozen or fixed in paraformaldehyde for necropsy analysis.
2. Symptoms of nematode infection: skinny, arched body or a failure to mate or feed.
3. Symptoms of TB infection: raised scales, open lesions, and wound spots.

Light and Temperature Control

1. 9 AM – 11 PM lights on; 11 PM – 9 AM lights off.
2. Air temperature: 28 °C, Water temperature: 28.5 °C.

Water Quality

The fish facility includes two independent, recirculating systems (Quarantine and Main systems). Fish facility water is passed through a charcoal filter and then through a Millipore reverse-osmosis unit for storage in a 50-gal holding tank. A ten percent water exchange occurs daily for each Z-Mod rack (Quarantine and Main systems) using an automatic timer. Water in the Z-Mod racks is passed through charcoal and paper filters and irradiated with ultraviolet light in a continuous cycle.

1. pH: 6.5 – 7.5. If the pH deviates from this range, adjust with the acid/base buffer.

2. Conductivity: 200 μ S. If the conductivity deviates from this range, adjust with the Equilibrium salt solution.
3. Ammonia levels: should be less than 2 ppm. If levels are greater than this, introduce fresh system water to dilute this waste product.
4. Nitrite levels: should be less than 1 ppm. If levels are greater than this, introduce fresh system water to dilute this contaminant.
5. Nitrate levels: should be less than 40 ppm. If levels are greater than this, introduce fresh system water to dilute this contaminant.

Daily Operating Procedures

1. Check for system leaks
2. Check pressure reading for clogged filters (both pre-system and system filters).
3. Check for clogged screens in individual fish tanks and clean of necessary.
4. Check conductivity levels and adjust if necessary. Enter values in the Zebrafish Facility Log.
5. Siphon the bottoms of tanks to remove any uneaten food or debris.
6. Evaluate fish health. Remove all dead fish from the tanks and isolate possibly sick fish. Record these actions in the Zebrafish Facility Log.
7. Feed fish at 11 AM and 4 PM on weekdays and at 12 PM on weekends (see below).
8. Set up new brine shrimp culture at 4-5 PM.
9. Lock fish room at end of work day.

Weekly Operating Procedures

1. Check ammonia, nitrite, and nitrate levels. Enter values in the Zebrafish Facility Log.
2. Check pH levels and adjust if necessary.

Monthly Operating Procedures

1. Change the system and RO filters as necessary.
2. Clean and autoclave fish tanks as necessary.
3. Clean UV inserts if necessary.
4. Check and replace particulate filter if necessary.
5. Check and replace charcoal filter if necessary.
6. Change the poly filter pad for each Z-Mod rack if necessary. The poly filter pad can be cleaned between changes.

Yearly Operating Procedure

1. Change UV sterilizer bulbs and inserts.

Feeding Adult Fish

Fish are fed twice a day on weekdays. On weekends, twice a day is ideal, but once a day will suffice. Brine shrimp is the primary adult fish food. Add enough brine shrimp that can be consumed in 5 minutes.

Adult fish should also be fed supplemental foods. On even days fish should be fed the flake food (Aquatox); on odd days, the brown pellet food (Aquatic Habitat's Adult Zebrafish Food). Both are stored at 4 °C. Add enough food that can be consumed in 5 minutes.

On weekdays, feed the fish brine shrimp in the morning and afternoons (11 AM and 4 PM). Add the supplemental food in the afternoon (4 PM). *Do not feed fish in the mating tanks*—these will be fed upon their return to the Quarantine or Main system racks. On weekends, feed brine shrimp using the entire yield from the 2 x 1.5-L cultures, then supplement with flake or pellet food.

Feeding Baby Fish

Embryos are fed larval food twice a day 5-6 days after fertilization. Larval feeding continues until 14 days after fertilization, after which it is supplemented with live brine shrimp. Fish that are over 21-days old are subjected to the adult fish feeding schedule. At this point the fish can be permanently housed in the main or quarantine systems.

Until fish are permanently housed into the main or quarantine systems, they should be placed in the appropriate system for 30 minutes each day to flush out contaminated water. A convenient time to do this is while you are waiting for the brine shrimp culture to settle (see below). After 30 minutes, the tanks should be removed from the water systems and fed larval food.

Brine Shrimp Production

1. Dissolve 45 g of Instant Ocean salt in 1.5 L MilliQ water and add to a 2-L brine shrimp hatchery. Add 7.5 mL of brine shrimp eggs (stored at 4 °C in a sealed can). Repeat for second hatchery.
2. Vigorously agitate with air bubbling for 48 hours.
3. Stop air flow for 30 minutes to allow unhatched eggs, live brine shrimp, and egg casings to separate.
4. The unhatched eggs (dark brown material) compose the most bottom layer, and should be removed.
5. Live brine shrimp (orange material) compose the next layer and should be collected by filtration into a brine shrimp net. The collected shrimp should be washed with and resuspended in *Main system water*. Pool shrimp from both 2-L hatcheries. The shrimp suspension should be kept in the Brine Shrimp Stock 500-mL bottle and amounts needed for the Quarantine and Main Systems should be transferred to the appropriate squirt bottles for the first feeding of the day.
6. Approximately 4 mL of this brine shrimp suspension should be fed to 8 adult fish (first feeding)
7. The remaining brine shrimp suspension in the Brine Shrimp Stock bottle should be aerated by bubbling until the second feeding. Transfer this solution to the squirt bottles just prior to the second feeding.

Setting up Crosses

1. Set up mating crosses about one hour after the second feeding.
2. Assemble Aquatic Ecosystems breeding tanks with plastic partitions and fill with system water.
3. Place 1-5 female fish in one compartment and an equal number of male fish in the other.
4. Keep the segregated fish in the climate- and light-controlled fish room to allow mating the next morning.
5. Remove the plastic partitions to allow the fish to mate as soon as possible after the light comes on the next morning (9 AM).
6. Check for eggs every 30 minutes or so and collect them in separate Petri dishes.
7. Wash eggs extensively with egg water, using a mesh sieve to remove debris.
8. Place eggs in new Petri dishes, approximately 50 embryos/10-cm dish with about 30 mL of egg water.
9. Incubate eggs at the external 28.5 °C incubator to ensure properly staging. Remove dead or contaminated eggs regularly to ensure maximal survival rates.

Bleaching Embryos for Main System Aquaculture

Embryos to be transferred to the Main System *must* be bleached prior to their introduction. Procedures for this process can be found in Nüsslein-Volhard's Zebrafish book, which is located in the laboratory.

Tank Labeling

All aquaculture tanks containing zebrafish larvae and adult fish should be clearly labeled using removable tape. Wildtype fish and transgenic fish should be segregated (except for crosses) and should be housed in separate aquaculture tanks. Tank labels should include information on the fish line (wildtype, transgenic, or mutant strain) and the date of birth. Please note that the use of transgenic fish is restricted by the California Department of Fish and Game. No transgenic fish should ever leave the lab without proper authorization.

Tank Sanitization

Aquaculture and mating tanks should be sanitized between uses, particularly in preparation for Main System fish. The tanks should be soaked for 24 hours in a 0.6% solution of sodium hypochlorite and soaked for at least one hour in a 0.3% solution of sodium thiosulfate (to quench any residual sodium hypochlorite). The tanks should then be transferred to the Chen Lab dishwasher located in CCSR 3141 and washed using the normal cycle on sterilization mode. Sterilized aquaculture tanks should be stored in the designated laboratory shelves. Sterilized mating tanks should be stored on the carts located in the fish facility itself.