



TITLE: Daily Feeding Procedure for Zebrafish

SOP Category: Aquatic

RUAC SOP #: 5.04

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Effective Date: 6/17/2025

Approval:

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SCOPE:

This document describes the procedures to be followed for the daily feeding of zebrafish. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University facilities.

OBJECTIVE:

The objective is to describe the daily feeding procedure for zebrafish at their various life stages housed in the Research Tower rooms R-B13 and R-B19.

PROCEDURES:

Feed Frequency, Preparation, and Storage

1. Zebrafish are fed a combination of live brine shrimp and commercially available dry feeds (e.g., Tecniplast ZEBRAFEED, Skretting GEMMA, TetraMin Flake).
2. Feeding of zebrafish <5-7 days post fertilization (dpf) is not required as they subsist entirely on their yolk sac.
3. All zebrafish are fed at least twice on regular weekdays and once on weekends and university holidays/closures (or with Director approval).
4. Juvenile zebrafish (30-90 days old) are fed an additional optional third feeding on weekdays for advancing growth.
5. Decapsulated Brine Shrimp is prepared in-house monthly or can be commercially purchased (eg. Brine Shrimp Direct). Shrimp are hatched for 18-24 hours in aerated 25 ppt salt water before being fed out.
6. All feeds are stored in sealed containers in the refrigerator and marked with the expiration date.

Daily Procedure:

1. Record the room temperature and humidity on the log sheet for each room.
2. Record the fish system water temperature, pH, and conductivity information on the log sheet for each room (R-B13/18/19) (Monday–Friday). The correct ranges are

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displayed in green. Any parameter outside the correct range is highlighted in red and will trigger an alarm.

Display screen for R-B13/18 system:



Display screen for R-B19 system:



3. Report any alarms on the touchscreens to the Aquatic Supervisor or designee.
4. Confirm each tank has a steady stream of water flow, and nursery tanks have a constant drip flow.
5. Check fish health (See Daily Health Monitoring SOP).
 - a. Remove any deceased fish.
 - b. Wear gloves and remove the tank from the rack. Use a net to remove the fish and place them into a plastic bag, then into a brown paper bag. Flag the tank with a Post-it note recording the date and number of found dead. Write the number found dead in the "health" column of the log sheet. Take the bag to the carcass disposal area.
6. Proceed to the feeding procedure according to the weekday or weekend/holiday schedule.

Feeding Procedure:

I. Weekend/Holiday (Feed rooms RB13/19 dry feed 1x/day)

RB-13:

Adult tanks:

1. Fill squirt bottle to the line with reverse osmosis (RO) water and add two teaspoons of dry feed to it. Prepare the feed bottle immediately before use.



2. Gently swirl or invert the bottle, keep it well mixed, and prevent food from settling to the bottom as you feed.

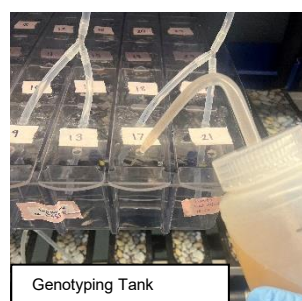
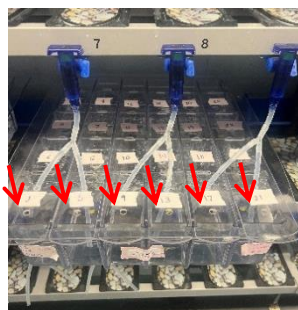
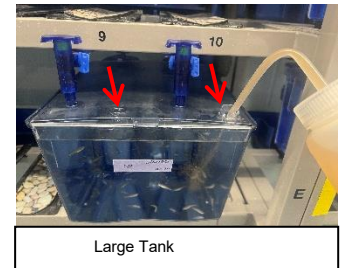
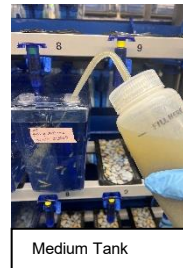
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3. Squeeze the bottle into the lid opening of each tank containing fish as follows:

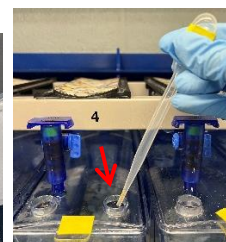
- Medium Tank (3.5 Liter):
 - 1 – 4 fish: one-second count
 - 5-13 fish – two-second count
 - 14-17 fish – three-second count
- Large tank (8.0-liter):
 - Two-second count both lid openings
- Genotyping tank (24-well single housing):
 - One-second count into each opening across the front row (red arrows).



4. Observe the previously fed tanks to ensure they are eating.
5. Repeat this process until all adult tanks have been fed.
 - a. You will need 3 to 6 bottles to feed the room, depending on tank counts.
6. Rinse the feeder bottle with RO water and place it in the drying area.

Nursery tanks (rack 10):

1. Use the pipette to stab into the container of dry larval feed (50-75 microns).
2. Dispense the food from the tip by squeezing the pipette bulb into the lid opening of each tank on the nursery rack (red arrows).
 - a. The fine powder will spread out on the top of the tank. Avoid dropping large clumps as they may sink to the bottom of the tank. Clumps may be broken up with the pipette before feeding.



RB-19 (Quarantine, Stand-alone rack):

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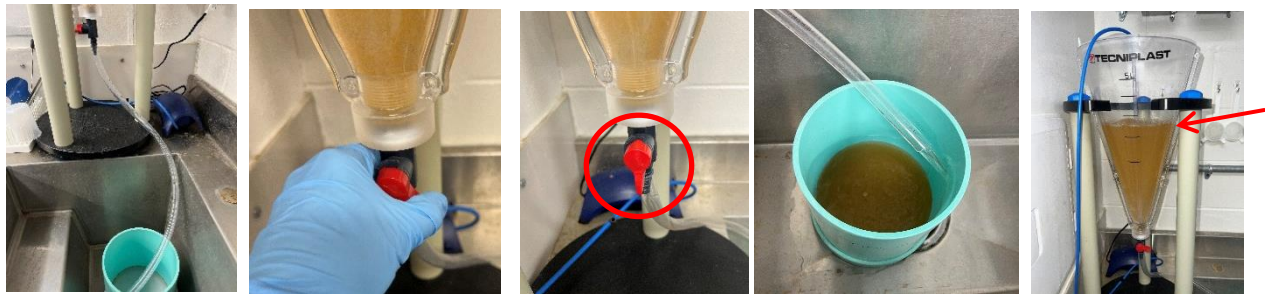
1. Adult tanks: Feed method based on rack occupancy.
 - a. One row or less – Add one 1/34 teaspoon scoop of dry feed from the conical tube directly into each tank.



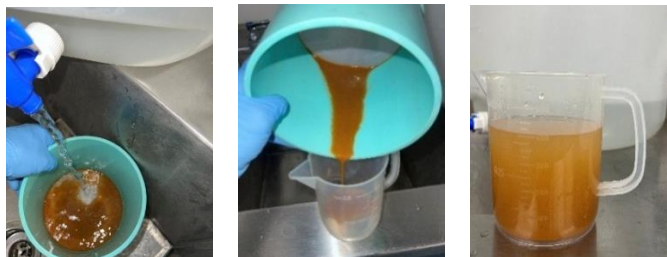
- b. Half rack (~25 tanks) - Fill squirt bottle halfway with RO water and add one teaspoon scoop of adult dry food.
 - c. Full rack (~50 tanks) - Fill squirt bottle to the line with RO water and add two teaspoons of adult dry food.
2. Nursery tanks: Repeat the method described above with larval food in the RB-19 refrigerator.

II. Weekday Feed (Feed 2x/day; AM and PM)

1. Collect the brine shrimp from the 5-liter hatcher in RB-13:
 - a. Collect half for the AM feeding and the other half for the PM feeding.
 - b. In the sink, collect shrimp by positioning the hose into the strainer and opening the valve (red circle) at the bottom of the hatcher to drain. In the AM, close the valve halfway after 2.5 Liters (red arrow).



- c. Rinse shrimp with RO water in the strainer to remove excess salt.
 - d. Transfer the shrimp into the pitcher using the RO water squirt bottle and fill to the ~400-500 mL line with RO water.



- e. Note: If brine shrimp is unavailable, use the dry feed procedure instead.
2. Fill one conical tube of brine shrimp with 20 mL (half rack) or 40 mL (full rack) to set aside for RB-19 fish.

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3. Go to nursery row in RB-13 with the brine shrimp pitcher and a graduated pipette. Starting at 12 days post-fertilization, add 0.5mL (less than 10 fish) to 1.0mL (higher density) per tank.
 - a. Also feed all nursery tanks dry feed as described above.
4. Determine how many bottles are needed to feed the adult fish tanks in R-B13 and divide the collected brine shrimp evenly among the bottles required, for example:
 - a. If three bottles are needed, fill the brine pitcher to 450 mL and divide 150 mL into each bottle. If four bottles are needed, fill to 400 mL and divide into 100 mL each, etc.
 - b. Stir the pitcher to keep the mixture mixed and add the first portion of shrimp to the feeder bottle.
 - c. Fill the rest of the bottle to the line with RO water.
 - d. Add one teaspoon of dry feed.
5. Gently swirl or invert the bottle to mix and prevent the shrimp from settling to the bottom as you feed.
6. Squeeze the food bottle into the lid opening of each tank type for the specified amount of time, measured in seconds, as described above.
7. Observe the previously fed tanks to ensure they are eating.
8. Repeat this process until all adult tanks have been fed.
9. Set up brine shrimp hatcher for the next day (Monday–Thursday):
 - a. First, clean the hatcher by scrubbing any residue from the walls and then rinse it out with RO water.
 - b. Measure a beaker of 130 mL of Instant Ocean Salt and add it into the empty (clean) hatcher.
 - c. Fill hatcher with RO water from the carboy using the 2-Liter beaker to transfer.
 - d. Add the air line (make sure the hose is securely attached to the air source).
 - e. Once salt is dissolved (stir to help it along), add one prepared tube of decapsulated shrimp and place the hatcher lid on with the air stick in place



10. Take the shrimp tube from step 2 to RB-19.
 - a. If there is a nursery row, give 0.5- 1.0mL per tank by pipette.
 - b. Adult tank feed method based on rack occupancy:

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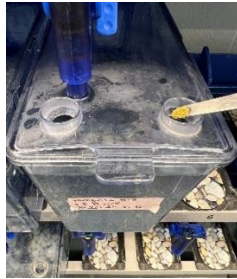
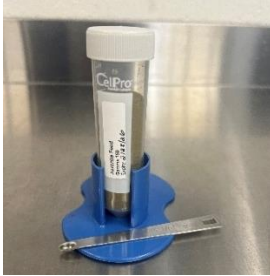
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- c. One row or less – Add 2.0mL brine shrimp by pipette, one 1/64 teaspoon scoop of dry feed from the conical tube directly into each tank
- d. Half rack (~25 tanks) - Fill squirt bottle halfway with RO water and add 20 mL of brine shrimp plus a half-teaspoon scoop of adult dry food.
- e. Full rack (~50 tanks) - Fill squirt bottle to the line with RO water and add 40 mL of brine shrimp and one teaspoon of adult dry food.

III. Juvenile/Nursery Optional Third Feed (Late PM, RB-13 Rack 9)

1. Add one 1/64 teaspoon scoop of juvenile dry feed (~150 micron size) from the conical tube directly into each tank on the juvenile rack.



2. Feed the nursery area as described (rack 10).
3. Log in the room sheet third optional feed column.