

Standard Operating Procedure for Zebrafish Husbandry

Purpose

To maintain consistency and quality in the husbandry of the zebrafish maintained at FLSC, despite being performed by multiple staff members, this document has been developed.

Basic fry procedures

1. Every Monday
 - a. Collect fry from incubator (eggs spawned on Thursday and Friday).
 - b. Move newly hatched fry to beakers or small tanks.
 - c. Place in 516 or 513 depending on PI or experimental requirements
2. Every Friday
 - a. Collect fry from incubator (eggs spawned on Tuesday and Wednesday).
 - b. Move newly hatched fry to beakers or small tanks.
 - c. Place in 516 or 513 depending on PI or experimental requirements
3. Beakers, tanks and water baths
 - a. All fry containers are labeled with strain and the date eggs were laid.
 - b. Fry are placed on designated racks in 513 or on the counter in 516 according to PI and age. Older fry are shifted as new fry arrive.
 - c. Some strains require a water bath due to strain or gene K/O.
 - d. Beakers and small tanks in 516 and 513 are checked daily for dead fry.
 - e. Fry containers are syphoned as needed or at least twice a week.
 - f. System water is added to beakers and small tanks in 516 and 513 three times a week.
 - g. Water baths are checked and filled three times a week with RO water.
 - h. All beakers and small tanks with fry are cleaned weekly (see room chart).

Moving fry to circulating rack systems

1. Fry are moved according to size and ability to consume the appropriate food.
2. Typically at 4-5 weeks from hatching, fry are moved to a circulating system rack in a designated room.
3. Bar codes are generated for each new tank that is on the system.
4. Excel spreadsheets may be used to keep track of large family groups, strains, room placement and rack location.
5. When placing a new tank on the system, use the appropriate size of screen baffle or plug mesh to ensure fry cannot pass through. Be sure to turn on the water to each new tank.

Adult Husbandry

1. All adult tanks are syphoned weekly.
2. All breeding tanks are syphoned at weekly or more often as needed.
3. Tank lids are wiped down weekly.
4. Troughs are wiped down weekly.
5. All tanks are changed every 8 weeks.
6. Tanks exhibiting algae prior to 8 weeks will be changed early.
7. Screened drain plugs and baffles are changed every 8 weeks or more often as needed.

General System Maintenance

1. Cleaning or replacing pre-filter pads on system reservoir tanks are performed twice a week for each system room according to the room calendar.
2. Check all systems for leaks or potential problems.
3. Record all life systems daily for each room on the record sheet. The stand-alone system in 022 has a separate record sheet.
4. Parameters that are monitored are pH, Conductivity, Filter pressure, Temperature.

5. Zebra fish systems are tested weekly using a commercial fresh water test kit for levels of Ammonia, Nitrate, and Nitrite.
6. As parameters for pH and conductivity increase or decrease, a pre-weighed amount of salt or sodium bicarbonate is added.
 - a. To increase conductivity, add sea salt.
 - b. To increase pH, add sodium bicarbonate.
 - c. To decrease conductivity, add RO water, effectively diluting the system.
 - d. In 022 and 516, the dosers auto fill RO water into the container.
7. At minimum, once a week add 500g of Sodium Bicarbonate and 1000g Sea Salt to all system room doser containers.
8. Some systems may need additional salt or sodium bicarbonate throughout the week.
9. All other dosers are manually filled with RO water which is typically a daily event.
10. Calibrate pH probes monthly.
11. System alarms are tested once a month. Notify the office prior to performing the alarm system test.
 - a. To simulate a low water alarm, hold a float down in a reservoir tank until system alarms.
 - b. Alarms are silent, calls and emails are generated to management.
12. System maintenance is performed annually by an outside contract for replacement of UV lights, pH probes, and conductivity probes. Maintenance contractor will also check the temperature probe and will troubleshoot and check for any other issues.

Equipment Maintenance

1. Shrimp sprayers are rinsed with tap water after each use and sanitized monthly using a 10% bleach solution.
2. Nets are cleaned once a month, which are ran through the cage wash unit.
3. Shrimp hatcheries are rinsed with RO after each use and sanitized monthly using a 10% bleach solution.
4. The salt tank is scrubbed and rinsed in place once a year.
5. The salt pump is checked or changed every 3 months by an electrician. A spare pump should always be available.
6. Water baths are cleaned every 6 months or more often as needed with a 10% bleach solution.
7. Food containers are cleaned monthly and require labels and expiration dates.
8. Counters are cleaned weekly with a quatricide solution with a 10 minute contact time or Rescue® with a 1 minute contact time.
9. Floors are mopped weekly with a quatricide solution with a 10 minute contact time.
10. Room photoperiods are generally 14:10, 14 hours light - 10 hours dark.
11. Euthanasia solution, Tricaine-S (MS-222), is mixed at 1-3 grams per liter. Euthanasia solution is discarded at 3 months from the time of mixing. Discard if the solution turns yellow. The storage bucket is cleaned after discarding the solution and prior to remixing. Euthanasia solution requires secondary labels and expiration dates.
12. Bleach solution is used for most routine sanitation in the zebrafish area. To mix 10% (1:9) add 250 ml (1 cup) household bleach to 2250 ml (9 cups) of tap water.