

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

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TITLE: Zebrafish Care and Feeding
SCOPE: Animal Care Personnel
RESPONSIBILITY: Facility Manager and Technical Staff
PURPOSE: To Outline the Proper Procedures for the Care and Feeding of Zebrafish.

I. PROCEDURES

1. Receiving

- a. Zebrafish are shipped/transported in sealed, water filled, plastic bags with oxygen added. Upon arrival shipping bags are inspected for signs of damage and shipment accuracy.
- b. Inspect fish for any signs of stress, trauma, or mortality that may have occurred while in transit.
- c. Fish and fish embryos from approved vendors are introduced into an appropriate size tank in the Quarantine room.
- d. Fish are acclimated to the aquatic system within 24 hours of arrival by emptying the fish into an empty tank connected to the system.
- e. Fish arriving with health concerns, or found sick, are isolated from healthy fish by placing them in their own isolation tank outside the system and the Facility Manager, PI and Clinical Veterinarian notified as needed.
- f. Individual shipments are identified by source, date of arrival, and any pertinent information.
- g. New tank numbers are recorded at time of arrival on **Per Diem Sheets**.
- h. Only bleached embryos are transferred out of Quarantine into the Main housing room.

2. Housing

- a. As a rule of thumb, fish should be housed in the Tecniplast ZebTec Zebrafish housing system. The maximum density is 5 adult fish per liter of water.
- b. A net is utilized for catching fish that must be removed from a tank.
- c. Water conditions and quality for Zebrafish are maintained as follows:

Tecniplast ZebTec™ Water Quality Guidelines			
Parameter	Range	Preferred	Confirmed
Temperature	72-82°F	78°F	Daily
PH	6.8-7.8	7.4	Daily
Conductivity	400-600 µS	550 µS	Daily
Alkalinity	50-200 ppm	170 ppm	Weekly
Hardness	0 dGH	0 dGH	Weekly
Ammonia	0	0	Weekly
Nitrite	0-25 ppm	0	Weekly
Nitrate	0-40 ppm	10 ppm	Weekly

- d. Room light cycle, 14 hours light (e.g., 9 AM till 11 PM) /10 hours dark cycle.
- e. Housing access is limited to IACUC certified personnel only.

3. Feeding

- a. Zebrafish are fed a combination of live feeds and commercial processed dry feeds (e.g., Tecniplast ZEBRAFEED 200-400, ZEBRAFEED 100-200).
- b. Zebrafish should be fed twice daily by adding enough food to each tank so that food is available to all fish and nearly all food is eaten within 10 minutes.
- c. When feeding brine shrimp, use freshly harvested shrimp. Collect shrimp into a pipette while trying to avoid collecting any unhatched eggs into the pipette.
- d. When hand feeding, feed approximately one 1.5 ml pipette of shrimp per 10 adult fish. This can vary depending on the size and number of fish in the tank.
- e. Place pipette in the top of the tank and dispense about half the contents into the tank.
- f. Wait for the fish to consume most of the shrimp then dispense the remaining shrimp into the tank.
- g. Rinse pipette after each use. Place unused shrimp in the refrigerator for future use
- h. Record feeding on the ***Room Status Sheet***.

4. Daily Duties

- a. Conduct daily monitoring of zebrafish morbidity and mortality.
 - i. Removing sick fish from the population is our most effective means of detecting pathogens and adverse environmental conditions, preventing spread of disease, controlling morbidity, and ensuring a high level of fish welfare.
 - ii. Observations of sick fish and communications must be noted on the Room Status Sheet and the Facility Manager's Animal Health and Environmental Concern Form. Tanks with health concerns are flagged with a red sticker tab.
 - iii. Remove Fish exhibiting the behavioral and physical signs below:

Behavioral Abnormalities	Physical Abnormalities
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Fish at surface or near water inlet	Color change
Rapid breathing/opercular movements	Weight loss
Sluggish movements/lethargy	Exophthalmia/pop-eyes
Flashing/rubbing on tank surfaces	Distended abdomen
Circling, twirling, spinning	Skeletal deformity
Loss of equilibrium	Mass/swelling
	Hemorrhage/redness
	Gas bubbles
	Protruding scales
	Fin erosion or lesion
	Skin ulceration or lesion

- b. Place dead fish in a cooler at +4°C for 48 hours and lab informed.
- c. Feed Zebrafish twice daily as outlined above.
- d. Check the ZebTec system and verify system is operating properly using the checklist included on the **Room Status Sheet**, refer to the Tecniplast ZebTec™ User Manual for more detailed information.
- e. Inspect display and record the temperature, pH, and conductivity.
- f. Visually inspect the sea salt and bicarbonate tanks (e.g. refill as needed).
- g. Inspect the system. Additional maintenance schedules are conducted as needed.
- h. Assure no investigator's supplies or trash is left in the room.
- i. Check supplies (e.g., soap, paper towels, etc.).
- j. Clean counter/sink area and wipe down with disinfectant as needed.
- k. Complete **Room Status Sheet**.
- l. Sweep floor as needed and empty trash.
- m. Mop floor with AQUALIFE Multi-Purpose Cleaner dilution with water only.
- n. Adult fish that are sick and need to be euthanized: Contact lab to inform them about sick fish. If lab requests Comparative Medicine euthanize, immerse fish in buffered (pH 7.0 -7.4) Tricaine MS-222 at 250 mg/liter for 30 minutes following loss of rhythmic opercular movement. Zebrafish fry 4 to 14 days post fertilization (dpf) should be exposed for at least 20 additional minutes following loss of opercular movements. For embryos up to 7 days of age, immersion in diluted sodium hypochlorite solution (bleach at 6.15%) is acceptable. Always wear nitrile gloves, and safety glasses when handling Tricaine (work in fume hood when using Tricaine powder). Stock solutions can be frozen and stored for up to six months and must be labeled with an expiration date.

5. Weekly Duties

- a. Check NO₂
- b. Check NO₃
- c. Check NH₃
- d. Spot clean tanks
- e. Check water levels
- f. Per diem recorded
- g. Check genotyping tanks and email lab if fish have been housed longer than one week.

6. Bi-Weekly Duties

- a. Check heating elements.
- b. Check Drum Filter
- c. Check UV lamp
- d. Check Conductivity probe
- e. Check pH probe

7. Semi-annual Duties

- a. Disassemble tanks and clean with tap water.

Caution: Use of chemicals and/or detergents can be detrimental to fish, refer to ZebTec user manual.

II. References

1. Tecniplast ZebTec Stand Alone Aquatic System user manual.

Approved:

Date: